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THE BACTERIA OF MILK FRESHLY DRAWN FROM NORMAL UDDERS *

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INTRODUCTION

In the early years of the science of bacteriology it was supposed that freshly drawn milk was sterile and would keep indefinitely if outside contamination could be prevented. The earliest recorded determinations of the bacterial content of such milk were made in 1891 by Schulz,³¹ who reported that the first milk to be drawn contained large numbers of bacteria and that the numbers decreased as the milking progressed. But the idea prevailed for a number of years that normal milk drawn under aseptic conditions was sterile and that bacteria found in freshly drawn milk were an indication of a diseased udder. It was a mooted question until the work of Moore²³ and Ward³³ definitely proved that the normal udder may, and usually does, harbor bacteria throughout the whole extent of lactiferous ducts, including the most minute ducts, where the milk becomes contaminated as soon as secreted. This work was confirmed by De Freudenreich.¹²

Since the fact was established that milk is already contaminated before it is drawn, several investigators have studied this flora to determine the numbers and kinds of bacteria to be found there. The results of these investigators will be briefly summarized.

Gorini¹⁴ made a study of the milk from 22 cows and found the number of bacteria varying from a sterile milk to 300,000 per cubic centimeter. The most common organisms were micrococci, which he divided into 5 types according to their growth on lactose, gelatin, and milk. In the majority of cases he found these types existing together. In the milk from 6 of the cows he found streptococci. He concludes: "The normal flora of the milk ducts of cows consists essentially of these types of cocci, among which there are always some capable of changing the milk with the same rapidity as the real lactic ferments."

De Freudenreich¹³ found a varying number of bacteria with a great predominance of liquefying micrococci, some of which formed yellow, and some white colonies. He also found non-liquefying micrococci, a non-liquefying bacterium which he did not describe, and in the case of a few cows, *Bacterium lactis acidii*. This author noted that the bacteria which multiply in the udder

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are forms which are able to withstand the bactericidal properties of the milk and tissues. He states that "these bacteria appear to be quite inoffensive."

Harrison and Savage¹⁹ concluded that the flora of the udder is limited, and aside from that of the foremilk, which seems subject to a certain amount of change, is practically confined to a few species of white and yellow cocci, among which *M. albidus* and *M. varians* predominate. These organisms produce acid slowly in milk. *M. acidilactici* was found a few times. It sometimes produces acid enough to curdle milk.

Harding and Wilson¹⁸ studied more than 900 samples of milk taken with precautions against contamination. They found an average content of about 500 bacteria per cubic centimeter. Only 8% of the samples had a germ content of more than 1000 per cubic centimeter. Seventy-five percent of the cultures were classed as micrococci with *M. albidus* and *M. varians* occurring most frequently. Only 2 streptococcus cultures were found, and they were not typical strains. Several varieties of *Bacterium* were recorded under *Bacterium lactis*, with variety names *brevis*, *flocculus*, *citronis*, *nonacidi*, *aureum* II, *Connii*, and *acidi*.

Only one type of udder bacteria has been much discussed in the literature in its relation to sanitary milk. Various investigators have demonstrated the presence of streptococci in large numbers in milk coming from cows which have been affected with mastitis. Streptococci have also been found in milk coming from apparently healthy udders. In 1904, Petruschky²⁷ announced as a result of his investigations that the high infant mortality in summer was caused by streptococci which he found in milk in large numbers. Since then there has been considerable discussion about the significance of streptococci in milk in relation to disease. This discussion has been greatly confused by the similarity and apparently close relationship between the *Streptococcus pyogenes* of inflammatory processes and *Streptococcus lacticus* (Kruse) or *Bacterium lactis acidii*, as it is commonly called, the ordinary milk-souring organism which always gets into milk from external sources. In the opinion of many bacteriologists, Petruschky mistook *Streptococcus lacticus* for *Streptococcus pyogenes*.

In recent years a new interest has been directed to the presence of streptococci in milk because of several sore-throat epidemics, most notably the Chicago epidemic in 1911-12, which were caused by streptococcal infections, were shown to be milk-borne, and were traced to infected udders.

Hastings and Hoffmann²¹ studied the milk of two cows throughout one or two lactation periods in each case. These cows had shown abnormally high counts in a preliminary investigation. One averaged 38,000 and the other 30,700 bacteria per cubic centimeter. The high count in both udders was due to the presence of a streptococcus, altho neither animal was known to have had garget.

Baehr² examined 81 samples of milk in the vicinity of Düsseldorf. Part of the samples were from milk dealers and part were from individual cows. *Streptococcus pyogenes* was found in only 2 of all these samples, and in the case of 1 of these 2, the milk was from a diseased udder.

Sherman and Hastings³² obtained results very different from Baehr's. In their recent work they found streptococci in 0.01 c.c. of the mixed milk from 10 out of 12 herds and in 38.6% of the individual samples from 88 cows of 4 herds. One of these herds was well-known to the authors, and, so far as could be learned, there had been no case of udder-inflammation and no trouble had resulted from the use of this milk, which was sold largely for the feeding of children.

From all the work which has been done to show the constant presence of bacteria in freshly drawn milk it has come to be generally recognized that when the dairyman has done his best in the way of cleanliness, and when the most ideal conditions have been maintained from the time the milk is drawn until it is consumed, nevertheless it is contaminated from a source over which there can be little or no control. But with the exception of the streptococcus group very little is known about the nature of the bacteria of the udder. The literature is rich in comparative studies of strains of streptococci from different sources. The data showing the frequency of this type of bacteria in milk coming from healthy cows, however, are very meager. Certainly, in view of the fact that the number of streptococci in milk is frequently proposed as an index to its sanitary condition, this is an important consideration. Concerning the nature of the other types of bacteria in freshly drawn milk almost nothing is known. It has been assumed that they are inoffensive. But dairy bacteriology has progressed to a point where it is now desirable to have our knowledge based on something more substantial than assumption. It is a matter of especial interest to those who wish to obtain the best quality of milk for the feeding of infants or invalids, whether or not the bacteria normally present in the best of certified milks may ever be of a harmful nature, either because of their virulence or because of a possibility of their bringing about undesirable chemical changes in the milk.

The study here reported was undertaken to gain some knowledge of the frequency of the various types of bacteria normally present in freshly drawn milk, and to find out something more about the nature of those types concerning which little is known.

THE SAMPLES OF MILK STUDIED

All the samples of milk studied were taken from 5 herds of cows which are kept in modern clean barns. Three of the dairies belong to institutions in the vicinity of Washington, D. C., and 2 supply certified milk to the city of Chicago. Altogether, samples of milk from 161 cows have been studied. From some of the cows of Dairy 1, as many as 2, 3, or 4 samples were taken from different quarters of the udder, making 192 samples of milk studied in all. The samples were obtained at milking time after part of the milk had been drawn, to avoid contamination with bacteria which might have got into the teat after the last milking. The literature shows that such are washed out during the first part of the milking. The milker would draw a stream of milk into a sterile test tube which was immediately placed in ice water, where it was kept until plating was done. The samples from 3 of the dairies were taken in the afternoon and plated the following day. The samples from Dairy 1 were plated within an hour or two after they had been taken. Those from Dairy 2 were plated about 5 hours after the milk had been drawn.

METHOD OF OBTAINING THE CULTURES

It was the intention in this study to consider only cultures which represent the types of bacteria which can multiply in the udder and become localized there, and the selection of cultures was made always with this in view. No one method of plating and isolating was used. The study of one series of samples gave suggestions for improvements in the method which were carried out on the next series of samples. It will be unnecessary to describe all the steps taken. The method used in the study of the last series of samples will be described in detail, however, for this is the most successful method found for obtaining growth of all the types of bacteria of the udder.

Among the procedures discarded as useless were the plating of samples on gelatin, and the maintenance of anaerobic conditions for incubation.

The last series of samples was plated as follows: Two sets of plates were made for each sample, each set with 1:10 and 1:100 dilutions of the milk. Into one set of plates lactose agar was poured, and they were incubated at 30 C.; into the other set of plates serum agar was poured and they were incubated at 37 C. The serum agar was made by adding 10% of cow's blood serum to the melted agar just before pouring into the plates, at about 50 C. The blood serum had been obtained with precautions against contamination. It was preserved for use in test tubes to each of which had been added a few drops of chloroform. The tubes were kept in an ice-box. When the serum was to be used it was pipetted off. The small amount of chloroform retained in the serum was driven away by the heat of the melted agar, and did not interfere with the development of colonies.

After 6 days' incubation the colonies on the plates were counted and some were picked off for inoculation. In the case of most of the samples, all the inoculations were made from the serum agar. But if the colonies were very few in number, or if for any other reason it seemed probable that a more representative set of cultures for that particular sample would be obtained by making use of the lactose agar plates, colonies were also picked off them.

For each sample of milk about 20 cultures were made, all into litmus whole milk, with precautions to make several cultures from each type of colony on the plates and to avoid colonies which appeared to be contamination. After 2 days' incubation subcultures were made on infusion-agar slopes. These were incubated for 2 days, or longer if necessary, then the cultures were examined and most of them were discarded. Every odd culture from among the 20 was discarded as possible contamination or as representing a type too few in numbers to be worthy of further consideration. A single culture of each type representing 2 or more cultures from a sample of milk was selected for further study. The selection was made by comparing all the evidence available; namely, appearance of growth in litmus whole milk and growth on agar slopes. In this way sometimes a single culture or sometimes 4 or 5 cultures were obtained from a sample of milk for further consideration. A very rough estimate of the proportion of each type present in the sample could be obtained by recording the number of cultures represented by each one saved.

By this method of obtaining representative cultures, and by discarding every one concerning which there was any suspicion that it might have been a contamination after the milk was drawn, or that it might have made its way into the teat from external sources, it was made certain that those cultures which have been studied in detail and considered in this report represent types which multiply in the udder and occur in numbers large enough to receive consideration.

MILK SAMPLES FREE FROM CONTAMINATION

Among the plates made from the milk of each dairy, there were some which showed no growth on the 1:10 dilution, the lowest dilution plated. Other plates showed only 2 or 3 colonies. When there were so few colonies, it was thought likely that they came from outside contamination. Even tho they came from the udder they did not represent a large enough number of bacteria in the milk to receive consideration; therefore, they were discarded and the samples of milk from which such plates were made were considered free from bacteria.

Altogether, 31, or 16.1%, of the 192 samples were from quarters of the udder in which bacteria were not multiplying in numbers worth considering.

MORPHOLOGY OF UDDER BACTERIA

A microscopic examination was made of the cultures obtained by the method of selection described. The slides were prepared by making a smear from the condensation water of a 24-hour growth on an agar slope, or, if the culture developed slowly, of an older growth. The slides were stained according to the Gram-Weigert method (differing from the Gram-stain in decolorizing in a mixture of 2 parts of xylol and 1 part of anilin).

Drawings of all cultures were made with the aid of a camera lucida and with a magnification of 4800 diameters on the drawing board. According to their morphology the bacteria of the udder fall under 3 groups—streptococci, micrococci, and bacilli. These groups will be considered separately.

STREPTOCOCCI

A most important matter in the study of the streptococci of milk is the differentiation between the long-chain streptococci, which may be of a virulent type, and the saprophytic *Streptococcus lacticus* (Kruse). It is my opinion that such a differentiation can be made by the simple method here described, altho there are undoubtedly variations which do not conform to either of the two types.

The method used in this study for identifying the streptococci was that employed by the author¹⁰ in a previous work and used also by Sherman and Hastings³² in their recent work. The differentiation is made roughly by the characteristic growth in litmus milk.

Long-chain streptococci usually curdle the milk, and reduction of the litmus may take place after curdling, but the color is never completely reduced. With *Streptococcus lacticus* cultures the reduction of

litmus precedes the curdling and is complete beneath the sharply defined pink surface layer.

The cultures differentiated by this method were examined microscopically to confirm their identity with one type or the other. *Streptococcus lacticus* lacks the tendency to form long chains and some or all of the cells are elongated with tapering ends.

Frequency of Streptococci in Milk from Normal Udders.—From the 192 samples of milk studied, *Streptococcus lacticus* (Kruse) was isolated only once. It was discarded as contamination, as were all other cultures isolated only once. From these data the conclusion may be drawn that *Streptococcus lacticus* does not localize and multiply in the udder. This is in close agreement with the statement made by Rogers and Dahlberg²⁸ that 93% of their cultures of streptococci from the udder failed to reduce litmus.

Long-chain streptococci which failed to give the reduction of litmus characteristic for *Streptococcus lacticus* were found in the milk from all 5 of the dairies from which samples were obtained, in numbers ranging from a very few to 264,000 per cubic centimeter. The percentages of samples which contained streptococci were for the several dairies as follows: 3.5, 10.9, 12.5, 20, 27. Altogether, streptococci were isolated from 29, or 15.1% of the 192 samples.

No detailed study was made of the streptococcus cultures, because they are the subject of study by other investigators of this division.

MICROCOCCHI

Frequency of Micrococci in the Samples of Milk.—All investigators of the bacterial flora of the udder have agreed that micrococci occur there most frequently. The study here reported confirms this statement. They were found in numbers from a very few to 80,000 per cubic centimeter. The percentages of samples which contained micrococci were for the several dairies as follows: 39.3, 46.9, 47.5, 72.7, 75.7. Altogether micrococci were isolated from 113, or 58.8% of the 192 samples. The figures show merely the number of samples from which micrococci were isolated. They were undoubtedly present in more of the samples than the figures indicate, for in the case of samples containing great numbers of other forms, with comparatively few micrococci, they would sometimes fail to be isolated.

Cultural Methods for the Differentiation of Micrococci.—As a preliminary part of this study a large number of cultures of micrococci were submitted

to various tests, in order to learn which tests might be valuable for differentiation into groups. The determination of the amount of acid produced in broths containing the various carbohydrates and related substances which have been used by many investigators for differential media, was made, and of these only 6 appeared to be useful for the present study. They were dextrose, lactose, maltose, raffinose, mannite, and glycerin.

The decomposition of carbohydrates and related substances has come into common use for the classification of bacterial cultures, but with the exception of the reduction of nitrates, the formation of indol, and the liquefaction of gelatin, the action of bacteria on nitrogen-containing substances has been little used for purposes of classification. It seemed, however, that the ability to utilize simple nitrogenous compounds in synthetic media should give information concerning another phase of the physiologic activities of the cultures. Accordingly, inoculations were made into media containing as the only source of nitrogen simple compounds such as the amino-acids and the nitrate and ammonia compounds. Three of these were chosen as useful in this study of the micrococci. They were reduction of nitrates to nitrites and decomposition of asparagin and urea. Gelatin-liquefaction, pigment-formation, hemolysis, and action on litmus milk were also used as differential tests.

Pigment-Formation.—In order to determine the pigment-formation under uniform conditions, strokes were made on infusion-agar slopes and incubated for 2 weeks at 20 C. The growth was removed and spread thickly on a piece of white drawing paper. It was then compared immediately while moist with the color plates in Ridgway's "Color Standards and Nomenclature." This method for determining pigment-formation accurately was used in an earlier work²⁹ on bacteria of the colon type. The explanation of the symbols used to designate the color standards (see Table 1) is quoted from that paper:

"Colors are designated by letters as 'oy,' which indicates a color made up of 65 percent orange and 35 percent yellow; 'yoy,' 47 percent orange and 53 percent yellow; or 'oyy,' 25 percent orange and 75 percent yellow.

"Variations from the pure colors are obtained by mixtures of neutral gray. For instance, 17 oy is a pure color; 17'oy contains 32 percent neutral gray; 17''oy, 58 percent neutral gray.

"Finally, tints, designated by lower-case letters, are obtained by mixing varying proportions of white with the pure colors, and shades by mixing black. The tint nearest the pure color is designated by the letter b; that nearest the pure white by f; the shade nearest the pure color is designated by the letter i."

Gelatin-Liquefaction.—To determine the liquefaction of gelatin a drop of broth culture was spread on the surface of a tube of infusion gelatin, the surface of the gelatin was marked on strips of paper on opposite sides of the tube, and the cotton plug was paraffined to prevent evaporation. After 30 days' incubation at 20 C. the tubes were examined. If liquefaction had occurred it was measured and expressed in millimeters of liquefaction.

Hemolysis.—Blood-agar plates were made by adding 10% of defibrinated cow's blood to infusion agar at about 50 C. and pipetting 5 c.c. of this into each plate. When the agar had solidified streaks of the culture were made on it and the plate placed in the incubator at 37 C. Ordinarily those cultures showing hemolysis within 24 hours are designated positive and those that fail to show hemolysis in 24 hours are designated negative. But in this study it was desirable to show relationships between such positive- and negative-reacting cultures. Therefore the plates were incubated for 4 days, and slight hemolytic

action was recorded. The following scheme for recording hemolysis serves to show the relationship between active hemolytic cultures and those in which this power appears to be lacking.

Very active hemolysis in 24 hours.....	+	+	+	+
Moderate hemolysis in 24 hours.....	+	+	+	
Hemolysis in 2 days.....		+	+	
Slight hemolysis in 3 or 4 days.....			+	
No hemolysis				—

Nitrate-Reduction.—Nitrate-reduction was determined in a peptone-free solution. Ordinarily the reduction of nitrate is determined in a peptone broth. But on the general principle that the simplest possible medium is the best for bacteriologic determinations, the peptone-free solution was chosen as more reliable after a trial of both kinds of media. The results reported in this paper are, therefore, not comparable with other investigators' data on nitrate-

TABLE 1
CONSTANCY OF CULTURAL CHARACTERISTICS

Culture	Date Tested	Action in Milk	Dextrose	Lactose	Maltose
ab	Oct., 1913	Curdled in 14 days.....	4.60	3.75	3.45
	April, 1915	Curdled in 11 days.....	4.60	3.65	3.90
aj	Oct., 1913	Curdled in 4 days.....	3.75	1.50	3.30
	April, 1915	Curdled in 6 days.....	3.20	2.25	4.30
as	Oct., 1913	Acid, not curdled, in 14 days.....	4.00	3.30	
	April, 1915	Acid, not curdled, in 14 days.....	2.30	3.70	4.10
bh	Oct., 1913	Curdled in 4 days.....	4.45	4.25	4.35
	April, 1915	Curdled in 12 days.....	4.25	3.15	3.80
bu	Jan., 1914	Curdled in 14 days.....	3.90	3.95	2.55
	April, 1915	Curdled in 14 days.....	4.50	4.40	4.10
ci	Jan., 1914	Curdled in 5 days.....	2.90	2.15	2.55
	April, 1915	Curdled in 6 days.....	3.55	2.70	4.25
dx	March, 1914	Slightly acid in 14 days.....	3.90	3.90	3.30
	April, 1915	Slightly acid in 14 days.....	4.65	4.70	4.30
fj	April, 1914	Slightly acid in 14 days.....	4.05	3.80	3.25
	April, 1915	No change in 14 days.....	4.35	4.15	4.20

reduction by micrococci. The medium was made up as follows: 1 liter water, 1 gm. magnesium sulfate, 2 gm. dibasic potassium phosphate, 1 gm. dextrose, and 3 gm. potassium nitrate.

The reagent for detecting nitrates was made up as follows²²: (1) A solution of naphthalamine, 0.1 gm., in 20 gm. distilled water, was boiled, cooled, filtered, and 156 c.c. of dilute (1:16) acetic acid added. (2) Sulfanilic acid, 0.5 gm., was added to 150 c.c. dilute acetic acid. The two solutions were mixed in equal proportions before use.

The cultures were incubated for 7 days and then tested by adding a few drops of the reagent to 1 c.c. of the culture in a 10 mm. chemical test tube.

Decomposition of Asparagin.—Asparagin medium was made with 1 gm. magnesium sulfate, 2 gm. dibasic potassium phosphate, and 5 gm. asparagin in

1 liter of water. The cultures were incubated for 7 days at 30 C. and then tested for ammonia with Nessler's reagent.

Decomposition of Urea.—The urea was sterilized in a solid condition by heating for half an hour in a toluene oven at 105 C. This precaution is necessary, for urea in solution is broken down to ammonia during the process of sterilization. Tubes of salt solution (1 gm. magnesium sulfate and 2 gm. potassium dibasic phosphate in 1 liter of water) were sterilized and then a small piece of the sterile urea was placed in each tube. After 7 days' incubation at 30 C. the cultures were tested for ammonia with Nessler's reagent.

Fermentation Tests.—The media for the fermentation tests were made as follows: Beef extract, 4 gm.; peptone, 10 gm.; dibasic potassium phosphate, 5 gm.; test substance, 10 gm.; and water, 1000 gm.

After 7 days' incubation at 30 C., 5 c.c. of the culture were titrated in the cold with phenolphthalein as an indicator against 0.05 normal sodium hydroxid.

TABLE 1—*Continued*
CONSTANCY OF CULTURAL CHARACTERISTICS

Raffinose	Mannite	Glycerin	Liquefaction of Gelatin	Decomposition of Asparagin	Decomposition of Urea	Chromogenesis
.30	.75	.10		—	+	19 YOY, light cadmium
.05	1.00	.30		—	—	19 YOY, light cadmium
.85	1.25	1.40		--	+	15 YOb, capucine yellow
.90	.90	.70		--	—	15 YOb, capucine yellow
.15	.05	.40		—	—	21" OYY f, ivory yellow
.05	.30	.40		--	—	21" OYY f, ivory yellow
.20	— .15	1.35		—	+	21" OYY f, ivory yellow
— .05	.05	1.25		—	—	19' YOY f, cream color
.00	.10	.30		—	+	19' YOY f, cream color
.20	.20	.25		--	—	19' YOY f, cream color
.90	1.45	.80	17 mm.	—	+	17 OY f, pale orange yellow
1.00	1.10	.75	16 mm.	—	—	19' YOY f, cream color
.40	2.25	.65	20 mm.	—	+	17 OY b, deep chrome
.35	1.60	.65	15 mm.	—	—	15 YO b, capucine yellow
.15	1.85	.25		—	+	17 OY, cadmium yellow
.20	.95	.25		—	—	19 YOY, light cadmium

The acidity was recorded in cubic centimeters of n/20 NaOH with the acidity of a control tube subtracted.

Constancy of the Characteristics of Individual Cultures.—A question always of interest when cultures are classified according to certain characteristics is whether the characteristics are constant. A number of the cultures of micrococci were submitted to the tests a second time after being maintained a year or more on agar slopes. The results of the 1st and 2nd tests are given in Table 1 for comparison. Hemolysis and nitrate-reduction are omitted because these tests were not made until the cultures had been maintained on agar slopes for about a year. The gelatin-liquefaction of most of the cultures is omitted for the same reason.

As may be observed by a study of the table, the cultural characteristics, with the exception of urea-decomposition, were remarkably constant. The

cultures seem to have lost the ability to decompose urea, for most of them reacted positively in the 1st test and all reacted negatively in the 2nd test. It is possible that some error may have brought about this discrepancy and not too much stress is to be laid on it until more is known about this character test.

The tests on all the 6 fermentable substances were absolutely consistent in the two determinations. Most surprising, however, is the agreement of the 1st and 2nd determinations of pigment-formation. The pigment was matched to the same color plate in both tests in the case of 4 of the 8 cultures. For the other 4 cultures the differences in pigment between the 2 tests were very slight, as any one familiar with the color chart may observe from the table. For example, Culture *d.r* was matched to the deep chrome chart in the 1st test, and to the capucine yellow chart in the 2nd test. These two charts are of the same tint, as indicated by the letter "b." They differ only in the presence of a very little more yellow in the deep chrome chart. The difference is so slight that an untrained eye might easily confuse the two colors.

Chromogenesis has always been regarded as an extremely variable character. But the data in Table 1 show that this is a stable character in the micrococci from the udder when determined with accuracy after growth under standard conditions.

Classification of the Micrococci from the Udder.—Micrococci coming from the udder have been called by many names. *Micrococcus lactis* with the variety names *acidus*, *albidus*, and *varians* is found most frequently. Gorini^{15, 16} includes them in his group of *Micrococcus casei acidoproteolyticus*. He¹⁷ ascribes a certain connection between the vigorous development of this acido-rennet flora in the udder and certain inflammatory conditions. The literature contains no record of *Staphylococcus pyogenes* having been found in milk freshly drawn from normal udders, altho in their description of *M. lactis varians*, Conn, Esten, and Stocking⁵ state that the characters of this organism "agree essentially with those of *Staphylococcus pyogenes aureus*." They also state that they are "inclined to think that our type is the common *Staph. pyogenes aureus*."

A careful study of the micrococci from the udder showed that the great majority of them belonged to one type. The 185 cultures belonging to this type were arranged into groups according to their fermentation of the carbohydrates and alcohols used as test substances (Table 14). A study of the 8 groups thus classified showed that the other characteristics, action on litmus milk, hemolysis, liquefaction of gelatin, nitrate-reduction, decomposition of urea, and chromogenesis, varied within the 8 groups without any correlation. This method of classification, which has been found useful for the differentiation of other types of bacteria, has little or no value in the differentiation of this type of udder bacteria into varieties with the exception of the mannite test, to be discussed.

Three characteristics were evidently correlated; namely, hemolysis, pigment-formation, and mannite-fermentation. Attempts were therefore made to divide the cultures into natural groups. In differentiating the groups, hemolytic action which was evident in 24 hours was called positive and hemolytic action evident later than this was called negative. The formation of pure colors and the deepest tints indicated by "b" and "d" was considered positive pigmentation and the formation of paler tints indicated by "f" or "slight pigment" was considered negative. The production of 0.5 c.c. or more of 0.05 normal acid per 5 c.c. of culture medium was considered positive fermentation.

A tentative division of the cultures into 2 groups was made first on the basis of hemolytic action, then on the basis of mannite-fermentation, and finally on the basis of pigment-formation. All these methods of division gave the same 2 general groups, the one with positive hemolytic action, positive fermentation of mannite and positive pigment-formation, and the other group with these characteristics negative. A consideration of the groups according to the 3 methods of division showed that the type characteristics were most distinct when the division was made on the basis of mannite-fermentation. This classification was therefore adopted, and the data are presented in Table 2 and graphically in Figure 1. The group showing positive mannite-fermentation, positive hemolytic action, and positive pigment-formation was recognized as identical with the *Staphylococcus aureus* in medical literature.

Here it will be necessary to make a short digression to discuss the pyogenic staphylococci.

These are the organisms which cause furuncles, abscesses, suppuration, and septicemia, either alone or together with *Streptococcus pyogenes*. A deep yellow-orange pigmentation has long been associated with the most virulent type and accordingly this has been named *Staphylococcus aureus*. Andrewes and Gordon¹ studied the biologic characters of the staphylococci pathogenic for man, and found mannite-fermentation to be absolutely constant for all their cultures from pathogenic sources, whereas their cultures from other sources failed in this respect. They laid great stress, therefore, on the mannite test as of high differential value.

Staphylococcus albus is known as a less virulent variety which fails to form the yellow pigment.

In a recent work Dumas² studied 18 cultures from pathogenic sources, and found that all fermented dextrose, lactose, saccharose, maltose, and mannite but that only 2 fermented glycerin. The hemolytic power varied greatly. Nicolle and Césari,²⁵ in a consideration of the whole group of staphylococci from the skin and mucous membrane and from various suppurative processes, conclude that the non-chromogenic staphylococci are usually non-virulent.

The statements of the authors quoted may be taken as a general summary of the characteristics of the pyogenic staphylococci found in the literature.

In regard to the relationship between hemolytic action and virulence of staphylococci opinions differ from that of Flügge,¹¹ who states that strong hemolytic action and positive agglutination are a criterion for pathogenicity, to that of Bruck and Hidaka,⁴ who report that of 17 cultures isolated from cases of eczema only 35% formed hemolysin. The consensus of opinion, however, is that hemolysis may be taken as an indication of virulence, altho some virulent strains fail to hemolyze blood corpuscles and some non-virulent strains may be active in this respect. The virulence tests reported in the succeeding pages show that strong hemolytic action may be taken as an indication of virulence.

A consideration of the two groups of staphylococci as presented in Table 2 and graphically in Figure 1 shows that the group with positive fermentation of mannite, hemolysis, and pigment-formation agrees with *Staphylococcus aureus* in its generally accepted characteristics.

It will be observed that most of the characteristics of the staphylococci as shown in Table 2 and Figure 1, do not serve in any way to differentiate the 2 varieties. Decomposition of asparagin was negative for all the cultures of the group. Dextrose- and lactose-fermentation were positive for all the cultures. Glycerin-fermentation was sometimes positive and sometimes

TABLE 2
COMPARISON OF THE TWO VARIETIES OF STAPHYLOCOCCI FROM THE UDDER ON THE BASIS OF
MANNITE-FERMENTATION

	Mannite	Hemolysis		Dextrose		Lactose		Maltose	
		+	—	+	—	+	—	+	—
Number of cultures.....	+	49	46	95	0	95	0	95	0
Percentage.....	..	51.6	48.4	100	0	100	0	100	0
Number of cultures.....	—	14	76	90	0	90	0	85	5
Percentage.....	..	15.5	74.5	100	0	100	0	94.4	5.6

negative, but this reaction was not correlated with the other characteristics of the 2 varieties. The same may be said of the urea-fermentation. The action in litmus milk also appeared to have little significance.

The comparatively few cultures which failed to liquefy gelatin had this characteristic apparently uncorrelated with any other deviation from the type. And those cultures which gave a positive reaction liquefied the gelatin in varying degrees from a decided action to a very slight action. It is only a short step from slight liquefaction to no liquefaction. Therefore no especial significance is attached to the non-liquefaction of gelatin.

It must not be inferred that because the cultural studies discussed here have had no value in differentiating the staphylococci into varieties they have been useless. It is only by an investigation of the many possible physiologic activities of a large number of cultures that the general characteristics of a type may be learned.

It will be observed that of the cultures which failed to ferment mannite, almost all failed to hemolyze blood corpuscles and failed to form pigment in the majority of cases. The few cultures which failed to ferment maltose all belonged to this group.

Those cultures which fermented mannite also formed a pigment in most cases and about half of this group hemolyzed blood corpuscles. Practically all those cultures which fermented raffinose and those cultures which reduced nitrates belonged to this group.

The close relationship of the two varieties is shown by the many characteristics which they possess in common. The interrelation of the variety characteristics shows that no hard and fast lines can be drawn to differentiate them. For example, Culture *qb* (see Table 3) failed to ferment mannite and formed only a slight pigment, yet it hemolyzed blood corpuscles very actively. Many such interrelations between the two varieties were evident. Moreover, most of the cultures for which the hemolytic action was considered negative in differentiating the two varieties were not absolutely without hemolytic action, but possessed this function to a slight degree.

If earlier classifications of the staphylococci are accepted, the negatively reacting variety from the udder would be termed *Staph. epidermidis [albus]*. According to Andrewes and Gordon¹ *Staph. epidermidis [albus]* is differentiated from *Staph. albus* by its failure to ferment mannite. The Winslows³⁴ accepted this classification, but designated the type which fails to ferment mannite by the name *Albococcus epidermidis*. According to these same authorities, *Staph. albus*, or *Albococcus pyogenes* as the Winslows call it, is a white non-virulent variety which ferments mannite.

TABLE 2—*Continued*
COMPARISON OF THE TWO VARIETIES OF STAPHYLOCOCCI FROM THE UDDER ON THE BASIS OF
MANNITE-FERMENTATION

Raffinose		Mannite		Glycerin		Gelatin		Nitrate		Urea		Asparagin		Pigment	
+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—
32	63	95	0	45	50	85	10	15	80	71	24	0	95	77	18
33.7	66.3	100	0	47.4	52.6	89.5	10.5	15.8	84.2	74.7	25.3	0	100	81.0	19.0
2	88	0	90	57	33	79	11	3	87	60	30	0	90	35	55
2.2	97.8	0	100	63.3	36.7	87.7	12.3	3.3	96.7	66.7	33.3	0	100	38.9	61.1

After a careful consideration of all the data in regard to the staphylococci from the udder, it seemed most reasonable not to follow the generally accepted classification in which the failure to ferment mannite would place a culture in the *Staph. epidermidis* species, but to designate the negatively reacting variety as *Staph. albus*.

Because of the undoubted agreement of the micrococci of the udder with the staphylococci, the author suggests that the names *Micrococcus albidus*, *Micrococcus varians*, *Micrococcus acidus*, and all other names which have been applied to those micrococci of the udder which conform to this type, be dropped, since their use only leads to confusion, whereas simplicity in nomenclature is greatly needed.

Virulence of Staphylococci From the Udder.—According to the cultural tests, 95 of the 185 cultures of the staphylococci which were studied belonged to the aureus variety. It was a matter of importance, therefore, to find out whether these strains might be virulent. A number of rabbit inoculations were made to determine the virulence of the

cultures. (The writer wishes to express her thanks to Dr. George M. Potter, of the pathological division of this bureau, for making the animal inoculations and the postmortem examinations.) The cultures had been grown on agar slopes for periods varying from 2 to 16 months before the inoculations were made.

A part of the inoculations were made intravenously. According to Dreyer,⁷ inoculation into the knee joint is a more delicate test for virulence. A part of the inoculations were made by this method. The results of the rabbit inoculations are given in Table 3. The mannite-fermentation, hemolysis, and chromogenesis of the cultures, characteristics of interest in respect to virulence, are tabulated there also. The data are arranged in the order of the virulence of the cultures.

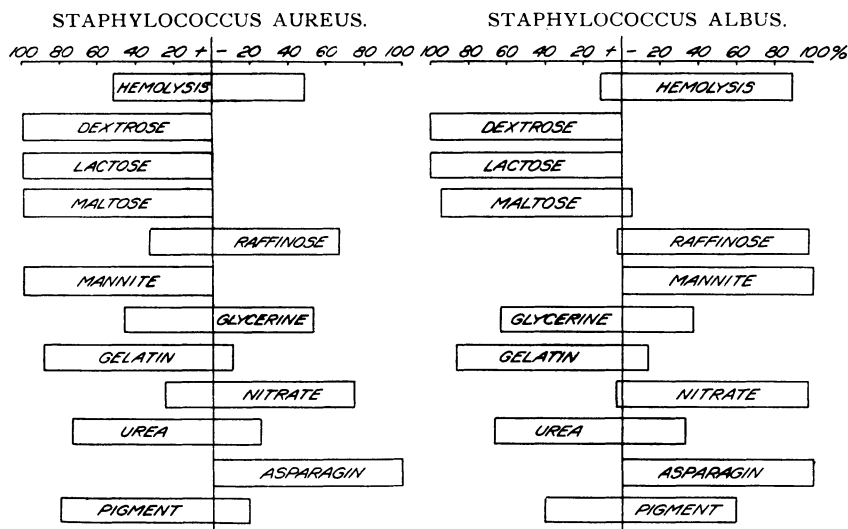


Fig. 1. Diagram showing the characters of *Staphylococcus aureus* and *Staphylococcus albus* cultures from normal udders.

Two of the cultures, *nq* and *ni*, proved to be exceedingly virulent. The inoculations were made in the afternoon, and death occurred during the following night. Six of the cultures, *nh*, *po*, *dx*, *dq*, *ib*, and *eb* produced severe local swellings, and the 2 animals which were not killed within a few days after inoculation died on the 11th and 14th days, respectively. Two other cultures, *mk* and *pc*, produced only slight swellings. The remaining 7 of the total 17 cultures inoculated showed no virulence.

TABLE 3
VIRULENCE OF STAPHYLOCOCCI FROM THE UDDER

Culture	Mannite Fermentation	Hemolysis	Chromogenesis	Result of Intra-articular Inoculation	Result of Intravenous Inoculation	Recovery of Organism
nq	1.85	++++	17 OY b, deep chrome		(1) Died within 18 hours (2) Died in 15 hours	No postmortem Cultures obtained from thoracic fluid and spleen
ni	1.60	++++	17 OY, cadmium yellow	Died within 18 hours	 Died within 18 hours	No postmortem Cultures obtained from heart's blood and spleen
nh	1.85	++++	17 OY b, deep chrome	Joint badly swollen on 2nd day. On 6th day rabbit killed. Joint and surrounding muscle purulent		Culture obtained from the joint
po	2.05	++++	17 OY b, antimony yellow	No swelling on 2nd day. On 6th day killed. Joint and surrounding muscle purulent		Culture obtained from the joint
dx	2.25	++++	17 OY b, deep chrome	Joint badly swollen on 3rd day. Rabbit died on 14th day	 Very sick on 3rd day. Slowly recovered. Killed on 19th day	Failed to obtain culture from heart's blood and spleen No growth from inoculations from heart's blood and spleen
dq	1.30	++++	17 OY, cadmium yellow	Joint swollen on 3rd day. Rabbit died on 14th day		No postmortem
ib	3.10	++++	19" YOY, honey yellow	 Joint badly swollen on 2nd day. Rabbit killed 6th day. Joint and surrounding muscle purulent	No effect	Culture obtained from the joint
eb	3.20	+++	17 OY, cadmium yellow	Joint badly swollen on 3rd day. Rabbit killed on 9th day		Culture obtained from the joint
mk	.05	+++	23" Y, strontian yellow	Joint slightly swollen and fever on 3rd day. A very little swollen on 8th day. Later, rabbit recovered		
pc	.20	++++	15 YO, orange....	 Joint slightly swollen on 3rd day. No swelling on 6th day. Rabbit killed on 9th day	No effect	No growth from inoculations from heart's blood, joint, and spleen

Tab 3 continued on p. 452.

TABLE 3—*Continued*
 VIRULENCE OF STAPHYLOCOCCI FROM THE UDDER

Culture	Mannite Fermentation	Hemolysis	Chromogenesis	Result of Intra-articular Inoculation	Result of Intravenous Inoculation	Recovery of Organism
df	2.10	++	17 OY b, deep chrome	No effect		
kz	1.15	+++	17 OY, cadmium yellow	No effect		
my	.10	++	23" Yf, marguerite yellow		No effect	
qb	.00	++++	19' YOY f, cream color	No effect.....	No effect	
qy	1.35	++++	15 YO d, orange buff	No effect.....	No effect	
rz	1.75	+++	17' OY b, antimony yellow	No effect.....	No effect	
sa	.20	+	13 OYO, cadmium orange		No effect	

In selecting the cultures for the virulence tests, a part were chosen because their active hemolysis and positive mannite-fermentation and pigment-formation indicated that they would be virulent. A number of the cultures were chosen for the purpose of determining the pathogenic properties when there was a variation in one or more of these characteristics.

It is an interesting fact that all 8 of the cultures which possessed sufficient virulence to cause serious results were mannite-fermenters which hemolyzed actively and formed a deep-colored pigment. But the non-virulent cultures and those showing slight virulence failed to give a positive reaction in one or more of the 3 positive characteristics of the aureus variety.

Frequency and Distribution of Virulent Strains of Staphylococci
 —It is impossible to estimate from the results with the 17 cultures which were inoculated what proportion of the 185 cultures possessed virulence. Since all the cultures which showed an unusually active hemolysis were inoculated into rabbits, it is probable that all the most virulent cultures were tested for their pathogenic properties. The two exceedingly virulent cultures, *nq* and *ni*, were both isolated from samples of milk from Dairy 4. Culture *nh*, with somewhat less virulence, was also from Dairy 4. The virulent culture *po* was from Dairy 5 and the other four virulent ones were from Dairy 1. The cultural tests indicated that none of the strains from Dairies 2 and 3 was virulent. Cultures *kz* from Dairy 2 and *mk* from Dairy 3 were chosen for inoculation as the most probably virulent of all the strains

from those two dairies. Culture *kz* proved to be non-virulent and Culture *mk* possessed only a slight virulence.

An estimation of the number of organisms of the virulent strains present in the milk from which they were isolated is given in Table 4. It may be noted that they vary from a very few to 80,000 per cubic centimeter.

TABLE 4
FREQUENCY OF VIRULENT STRAINS OF STAPHYLOCOCCUS AUREUS

Culture	Dairy	Estimated Number per c.c. of Milk
nq	4	50
ni	4	1600
nh	4	80,000
po	5	4000
dx	1	32,000
dq	1	270
jb	1	11,600
eb	1	30

Coccus Cultures not Included with the Staphylococci.—Not all the micrococci obtained from the udder were classed with the staphylococci. First among these to be discussed is a small group which was characterized by a negative reaction to most of the tests. Four of the 8 cultures gave too scant growth for the determination of pigment. These 4 cultures died after being maintained a few months on agar slopes. The other 4 cultures were re-inoculated in the test substances after a year's cultivation on agar slopes. At the time of the 2nd tests the cultures gave reactions similar to those of the staphylococci. It appears that the cultures had acquired the ability to ferment the test substances. But there is a possibility that some peculiar conditions which escaped observation might have prevented positive reactions in the 1st series of tests. Because of their peculiarity in the 1st tests these cultures have been omitted from the staphylococci to simplify the discussion.

Three other groups of micrococci from the udder gave reactions which were considered to differ from those of staphylococci sufficiently to separate them into distinct groups. One of these is presented in Table 5. The lack of any hemolytic action whatever, and the decomposition of asparagin, distinguish this group from the staphylococci. The group can not be identified with any described species, because its classification was made largely by means of its ability to decompose asparagin, a character heretofore not used in

classification. No name will be suggested for the group because too few cultures of it have been studied. Cultures of this group were isolated from only 4 samples of milk. These samples were all from Dairy 5.

TABLE 5
ASPARAGIN-DECOMPOSING, NON-HEMOLYTIC MICROCOCCI

Culture	Dairy	Cow	Action on Milk	Dextrose	Lactose	Maltose	Raffinose
sb	5	59	Acid, not curdled, in 14 days	3.35	3.05	2.05	— .65
sf	5	68	Acid, not curdled, in 14 days	3.50	3.30	1.65	— .10
sm	5	75	Acid, not curdled, in 14 days	3.25	3.00	2.05	.00
rr	5	56	Acid, not curdled, in 14 days	3.10	3.20	2.20	— .15

Another group of micrococci from the udder is presented in Table 6. The group is characterized by the non-liquefaction of gelatin and the ability to decompose both urea and asparagin. Dextrose is fermented but the ability to attack the other test substances is characteristically lacking. This group has been designated *M. luteus*, the name adopted by the Winslows³⁴ for cocci of this description. Three of the cultures do not conform with their description of this species in the positive

TABLE 6
MICROCOCCUS LUTEUS

Culture	Dairy	Cow	Action on Milk	Dextrose	Lactose	Maltose	Raffinose
af	1	1	No change in 14 days	4.25	.05	1.30	.25
ag	1	1	Acid, not curdled, in 14 days	6.55	2.35	.25	.10
ah	1	1	Acid, not curdled, in 14 days	4.45	2.90	.25	.10
db	1	27	No change in 14 days	1.70	— .05	.30	.05
fu	1	21	No change in 14 days	1.00	.95	.60	.00
py	5	30	No change in 14 days	1.20	— .20	.60	— .30

reaction in lactose broth, and 3 of the cultures disagree with their description in the reduction of nitrates. But all 6 of the cultures in Table 6 appear to belong to a related group, and the discrepancies mentioned are certainly not sufficient to give the group another name.

The last group of micrococci to be considered is presented in Table 7. This group is distinguished from all other udder organisms by the rapid and complete peptonization of the milk. Gelatin is liquefied, and dextrose is fermented. Maltose, mannite, and glycerin are usually

TABLE 5—*Continued*
ASPARAGIN-DECOMPOSING, NON-HEMOLYTIC MICROCOCCI

Mannite	Glycerin	Hemolysis	Liquefaction of Gelatin	Reduction of Nitrate	Decomposition of Asparagin	Decomposition of Urea	Chromogenesis
1.75	1.65	—	20 mm.	—	+	—	19" YOY f, cartridge buff
1.35	.70	—	25 mm.	—	+	—	19" YOY f, cartridge buff
2.10	2.20	—	25 mm.	+	+	—	19" YOY f, cartridge buff
1.95	.70	—	18 mm.	—	+	—	19" YOY f, cartridge buff

fermented. Hemolysis is very slight or negative. In respect to the nitrogenous test substances there is a considerable difference between the Cultures *ao*, *bf*, and *bc*, which reduce nitrates and decompose asparagin but not urea, and Cultures *oe* and *ov*; they do not reduce nitrate and they decompose urea but not asparagin. But because of the small number of cultures obtained it did not seem advisable to separate the group. This group could not be identified with any pre-

TABLE 6—*Continued*
MICROCOCCUS LUTEUS

Mannite	Glycerin	Hemolysis	Liquefaction of Gelatin	Reduction of Nitrate	Decomposition of Asparagin	Decomposition of Urea	Chromogenesis
.30	.35	+	—	—	+	+	Slight pigment
— .15	— .05	+++	—	—	+	+	19 YOY f, maize yellow
— .45	.10	+++	—	—	+	+	19" YOY b, mustard yellow
.85	.10	+	—	+	+	+	21 OYY f, Baryta yellow
— .05	— .15	—	—	+	+	+	21 OYY f, Baryta yellow
.05	.00	+++	—	+	+	+	17" OY, yellow ochre

vious description of micrococci, altho it seems probable that this group, as well as the staphylococci has been included by Gorini^{15, 16} in his *Micrococcus casei* *acido-proteolyticus*. The name *Micrococcus caseolyticus* is here suggested for this type characterized by the rapid

and complete peptonization of milk and by other characteristics as shown in Table 7. This type of organism is apparently widely distributed, for cultures were isolated from samples of milk taken at 4 of the 5 dairies.

TABLE 7
MICROCOCCLUS CASEOLYTICUS

Culture	Dairy	Cow	Action on Milk	Dextrose	Lactose	Maltose	Raffinose
ao	1	2	Peptonized in 5 days	3.85	.10	3.10	.40
be	1	2	Peptonized in 14 days	3.75	— .10	1.85	.30
bf	1	2	Peptonized in 12 days	4.00	.15	1.40	.40
qs	5	39	Peptonized in 14 days	1.85	.05	1.50	— .05
rc	5	46	Peptonized in 4 days	1.80	— .05	1.50	— .15
me	3	327	Peptonized in 4 days	5.45	.80	6.30	.50
oe	4	74	Peptonized in 6 days	3.00	3.10	.45	.15
ov	5	11	Peptonized in 5 days	1.20	3.40	1.65	.15

Localization of Peculiar Species and Strains.—An interesting fact observed in a study of the tables is the localization of peculiar species and strains. There are many examples of this, but only a few of the most striking will be mentioned. The isolation from 4 samples of one dairy of the non-hemolytic asparagin-decomposing micrococci (see Table 5) has already been noted. Among the staphylococci, the cultures from the milk of Dairy 2 which hemolyzed actively, all failed to liquefy gelatin. From the milk of Dairies 1 and 5 a number of cultures were isolated which had the peculiarity of fermenting all 6 of the test substances. From these same milk samples several cultures were isolated which were distinguished from all the others by the fermentation of only 2 of the test substances, dextrose and lactose. Also the cultures of the *M. luteus* type (see Table 6) were all from the milk of these two dairies. Interesting in this connection is the fact that Dairies 1 and 5 are only a few miles apart.

Localization of Various Micro-organisms in Certain Dairies.—Not only do peculiar strains of the organisms commonly present in udders become localized, but also organisms of an entirely different nature sometimes become established in the udders of many cows of a dairy. Moore and Ward²⁴ found a gas-producing bacterium in the udders of many cows of one herd. A streptothrix was found in 6 of the 28 sam-

ples of milk from Dairy 3. In one sample there were 5000 of these organisms per cubic centimeter. No study was made of the streptothrix.

A gram-negative bacterium which made on agar a luxurious growth

TABLE 7—*Continued*
MICROCOCCLUS CASEOLYTICUS

Mannite	Glycerin	Hemolysis	Liquefaction of Gelatin	Reduction of Nitrate	Decomposition of Asparagin	Decomposition of Urea	Chromogenesis
.95	.40	Slight	18 mm.	+	+	—	21" OYY f, ivory yellow
1.45	.35	Slight	20 mm.	+	+	—	21" OYY f, ivory yellow
.80	.10	Slight	20 mm.	+	+	—	21" OYY f, massicot yellow
.85	.70	—	25 mm.	—	—	—	19" YOY f, cartridge buff
.90	.65	—	22 mm.	—	—	—	19" YOY f, cartridge buff
4.25	1.25	—	60 mm.	—	—	—	23" Yellow f, marguerite yellow
.40	.50	Slight	40 mm.	—	—	+	15" YO, ochraceous orange
.25	.60	Slight	53 mm.	—	—	+	17" OY, yellow ochre

of a peculiar yellow shade was isolated from 4 of the 40 samples from Dairy 2. These cultures did not stand the artificial cultivation, all dying in the 3rd transfer before a detailed study of them had been made. But enough is known of them to warrant the statement that they were unlike any other organism isolated from the other samples of milk.

BACILLI COMMON IN NORMAL MILK

Besides the two groups of bacteria already discussed, which are commonly present in large numbers in milk freshly drawn from normal udders, namely, the streptococci and the staphylococci, a third group of organisms was found in this study to be common in the milk from all 5 of the herds from which samples were examined. These were bacilli of the *Bacillus abortus* type. No description of these organisms has been found in dairy literature. But Schroeder and Cotton³⁹ reported *B. abortus* to be common in milk. They determined its presence by inoculation into guinea-pigs. Whenever *B. abortus* was present in the milk, there was a slow development of lesions which were characteristic for this organism.

A preliminary report⁹ of the demonstration of these organisms by cultural methods has already been made. In this report, only one variety of bacillus from the udder was mentioned. It was described

under the name of *Bacillus abortus*, for it resembled the published descriptions of the bacillus of contagious abortion.

Further studies of the bacilli from the udder have been made, and it is now recognized that they do not all belong to a single variety but that there is a related group of bacilli common in milk from normal udders which will be called the *Bacillus abortus* type.

A special method for plating the milk is necessary to develop colonies of the *Bacillus abortus* type. A milk containing hundreds of thousands of these organisms might pass the ordinary routine bacteriologic analysis of milk without revealing a single colony. This explains why they never have been mentioned in dairy literature.

If milk samples are plated on serum agar, however, and incubated for several days, as directed in this paper under "Methods of Obtaining Cultures," the *B. abortus* type develops readily. When 2 sets of plates are made from samples of freshly-drawn milk, one on lactose agar and the other on serum agar, a significantly larger number of colonies on the serum-agar plates is rather certain evidence of the presence of bacilli of *B. abortus* type.

TABLE 8
FREQUENCY OF THE *BACILLUS ABORTUS* GROUP IN FRESHLY DRAWN MILK

Dairy	Number of Samples Examined	Number of Samples from Which <i>B. Abortus</i> was Isolated	Percentage of Samples from Which <i>B. Abortus</i> was Isolated	Lowest and Highest Numbers of <i>B. Abortus</i> per c.c. in the Samples of Milk
1	55	9	16.4	10 50,000
2	40	2	5.0	3200 4000
3	28	7	25.0	180 680
4	32	8	25.0	50 3000
5	37	19	51.4	40 7000

Frequency of the B. Abortus Group in Freshly Drawn Milk.—In discussing the frequency of this group in samples of milk, it is necessary to emphasize the fact that the figures presented in Table 8 are undoubtedly far below the actual numbers present in the milk. Particularly is this true in regard to the figures for Dairies 1 and 2, since the method described for determining the presence of the *B. abortus* type was developed gradually as the work progressed, and was not perfected until the last series of plates was made, for Dairy 5. The

figures may be regarded with increasing accuracy, therefore, from Dairy 1 to Dairy 5. Table 8 shows that bacteria of the *B. abortus* type occurred commonly in the samples of milk from all 5 of the dairies. Altogether, cultures were isolated from 45, or 23.4% of the 192 samples studied.

Description of the Varieties of B. Abortus from the Udder.—About one-half of the cultures of *B. abortus* isolated from the milk samples were identical in their behavior towards every medium into which they were inoculated. The most vigorous activity of these cultures was found in their hydrolization of butter fat. No description of this variety can be found in the literature. In fact, no recognition of the different varieties of *B. abortus* has been found. Therefore, the name *B. abortus* var. *lipolyticus* is given to the fat-splitting variety, to distinguish these cultures from those which do not have the fat-splitting property. A description of any one of the 33 cultures of this variety would apply equally well to all the others.

The cultures of *B. abortus* var. *lipolyticus* produce a very faint growth on meat-infusion agar slopes. The growth is in very small separate colonies, which are scattered over the whole surface of the slope if it happens to be moist at the time of inoculation, or the colonies are confined to a ribbon-like growth along the line of inoculation if the agar is comparatively dry.

Five percent of bile added to the agar gives a more vigorous growth. Ten percent of blood serum added to the agar gives the most favorable conditions for growth. On this medium the growth is vigorous and fairly rapid and in a few days the medium begins to take on a brownish tinge, which deepens with the age of the culture.

The *lipolyticus* variety illustrates admirably the peculiar preference of *B. abortus* for an oxygen supply at less than atmospheric pressure, which led Nowak²⁶ to devise special methods for incubating his cultures with the optimal conditions of aeration. When agar-shake cultures are seeded heavily throughout the entire mass, growth takes place in a thin layer, a few millimeters beneath the surface.

The cultures of *B. abortus* var. *lipolyticus* produce no effect on gelatin, nitrates, urea, or asparagin; they produce no acid from any of the carbohydrates and related substances which are commonly used for fermentation tests. In litmus milk from which the cream has been removed the growth is slight, and there is no apparent effect. But in litmus whole milk there is a rapid multiplication of cells, and slow development of acid, which is first evident in the cream layer. After a couple of weeks' growth at 37 C., the optimal temperature, the cream layer is converted into a firm, slightly granular, ill-smelling mass.

Cultures of the *lipolyticus* type were isolated from 33, or 12% of the 192 samples studied. This type was isolated from samples of milk taken from all 5 of the herds.

Seven other cultures were considered to be atypical *B. abortus* var. *lipolyticus* because they differed from the 33 type cultures in forming a heavier growth on the infusion-agar slope, which was of an apricot yellow color (Ridgway's "Color Standards and Nomenclature" Plate 19, YOY-b). Also, 4 of these cultures decomposed urea.

Another group of bacilli isolated from the samples of milk was considered a variety of *B. abortus* and is designated Variety *b*. These bacilli were similar

in morphology to all those included in the *B. abortus* type. Dextrose and maltose broths were rendered acid. There was no change of reaction in the other broths. This variety failed to break down butter fat. Growth on plain infusion agar was abundant and of a dirty yellowish color. In agar shake cultures growth occurred on the surface and in the upper 6 to 10 millimeters of the agar. The "diaphragm" growth rarely occurred. Asparagin, nitrates, and urea were usually decomposed. Gelatin was not liquefied. Since growth was vigorous on plain infusion agar, it was not especially favored by the addition of serum. Twelve cultures of this variety were isolated from Dairies 1, 3, and 5.

Still another variety, designated *c*, differed from Variety *b* only in rendering the broth media slightly alkaline and in liquefying gelatin in the case of a few cultures. Only 5 cultures of this variety, all from Dairy 5, were isolated.

Relationship Between the Varieties of the B. Abortus Group Found in Milk and Those From Pathogenic Sources.—It was a difficult problem to classify these bacilli common in milk from normal udders, for no complete descriptions of these varieties could be found. A few striking characteristics possessed in common with the bacillus of contagious abortion as described in the literature suggested the relationship to those organisms and a thorough study of the cultures from milk was made in order to compare them with the original descriptions of *B. abortus*, and with cultures obtained from pathogenic sources. The problem thus led to a study of the serum reactions of the bacilli from milk in comparison with those of cultures from pathogenic sources, and to a study of the pathogenic properties of the cultures from milk. These phases of the problem are being worked out in co-operation with the pathological division of this bureau and will be the subject of a separate paper.

A comparison of the organisms of this group, so far as the bacteriologic methods would go, is presented in a briefly summarized form in Table 9. In the 1st column is given a description of *B. abortus* found in the literature on the subject. The description given by Bang,³ who first described the organism in 1897, supplied most of the data for this column. Nowak's²⁶ description of *B. abortus* is quoted under two headings where Bang's description was lacking. The descriptions of these two investigators fully agree. In the 2nd column is given a description in summary of 24 cultures from pathogenic sources which were supplied by the pathological division of this bureau for this purpose. The cultures had been collected originally by several institutions in different states, and had all been under artificial cultivation for several months before the present study was made.

TABLE 9

A COMPARISON OF THE CHARACTERISTICS OF THE SEVERAL VARIETIES OF *BACILLUS ABORTUS*

	B. Abortus from Original Descriptions	B. Abortus from Pathogenic Sources	B. Abortus lipolyticus	B. Abortus Variety b	B. Abortus Variety c
Morphology	Small rods, the largest as long as the tubercle bacilli (Bang)	Slender rods, 0.8 to 1.5 microns in length	Slender rods 0.8 to 1.5 microns in length	Slender rods 0.8 to 1.5 microns in length	Slender rods 0.8 to 1.5 microns in length
Reaction in dextrose, maltose, lactose, raffinose, mannite, and glycerin broth	Alkaline broth is given an amphoteric or slightly acid reaction to Tournesol paper (Nowak)	Neutral broth is rendered slightly alkaline, except that a few cultures form a slight acidity in dextrose broth	No change.....	Dextrose and maltose broths are rendered acid. No change in other broths	Slightly alkaline
Decomposition of nitrogenous compounds		Nitrate, asparagin, and urea are commonly decomposed. Gelatin is not liquefied	Asparagin and nitrates are not decomposed. Urea is rarely decomposed. Gelatin is not liquefied	Asparagin nitrates and urea are usually decomposed. Gelatin is not liquefied	Asparagin, nitrates, and sometimes urea are decomposed. Gelatin is sometimes liquefied
Action in litmus whole milk		Rendered slightly alkaline	Acid is developed in the cream layer	In most cases, slightly alkaline. Sometimes no change	No change
Growth in agarshake	Growth in colonies is confined to a zone of from 10 to 15 mm. This zone lies about 5 mm. beneath the surface of the agar (Bang)	Good growth on surface. Sometimes a growth throughout a zone of several millimeters at the top. Rarely a "diaphragm" growth	Colonies confined to a thin layer a few millimeters beneath the surface	Like the growth of cultures from pathogenic sources. Sometimes colonies are scattered throughout the entire depth of the agar	Like the growth of cultures from pathogenic sources
Growth on plain infusion-agar slope	Separate colonies resemble rose-colored droplets reflecting a greenish tinge (Nowak)	Abundant compact growth. Color, 19"YOY, b and d (chamois and cream buff)	A few cultures resemble those from pathogenic sources. The majority give a scant growth in separate colonies	Like the growth of cultures from pathogenic sources	Like the growth of cultures from pathogenic sources
Growth in glycerin broth	A poor growth. A fine sediment is thrown down, made up of whitish grains (Bang)	Good growth which clouds the medium	Scant growth which does not cloud the medium. Sediment is made up of little granules	Abundant growth. The medium is usually clouded, but sometimes all growth is precipitated, leaving a clear medium	Like the growth of Variety "B"
Effect of serum in the agar	Growth is greatly favored (Bang)	Abundant growth without the serum	Growth greatly favored	Abundant growth without the serum	Abundant growth without the serum

The table shows that altho the morphology is fairly constant there is a wide variation in the varieties described under the different columns. There appears to be an essential difference between the characteristics of the organisms described by Bank and Nowak and those of the cultures collected from pathogenic sources in this country. Such differences can scarcely be accounted for unless it be assumed that the European investigators were working with another variety of the abortion bacillus.

The characteristics of the lipolyticus variety, in the 3rd column, agree in every respect with Nowak's description except that the color on agar slopes was not observed in the case of the lipolyticus variety. Most striking is the similarity of the peculiar growth of the lipolyticus variety in agar-shake cultures to that of Bang's bacillus. The colonies develop only in a thin layer a few millimeters below the surface of the agar. Bang identified *B. abortus* in mixed cultures by this one characteristic.

On the other hand, Varieties *b* and *c* resemble the cultures studied from pathogenic sources. Variety *b* differs from those cultures chiefly in its reaction in the different broths. Variety *c* bears a still greater resemblance to the cultures from pathogenic sources but 3 of the 5 cultures liquefied gelatin, and 2 of these 3 digested milk slowly.

Significance of the B. Abortus Type in Milk.—Aside from the interest due to an organism which is commonly present in milk and which is closely related to the pathogenic *B. abortus*, this type is important because of its biochemical activities. The ability of the cultures of the lipolyticus variety to form acid in whole milk has been mentioned. Since no acid was formed in milk from which the cream had been removed, it was reasonable to suppose that the acid was formed by the breaking down of the butter fat. Accordingly chemical determinations of the volatile acids formed in milk by cultures of the acid-producing type were made. (Chemical analyses were made by Dr. J. N. Currie, of the dairy division.) Culture *qj* incubated for 15 days in 375 c.c. of 20% cream on distillation to 1000 c.c. with steam gave 11.02 n/10 c.c. more of volatile acids than did the control flask.

Five other cultures were grown in 750 c.c. whole milk for periods varying from 2 to 3 weeks. The cultures were made slightly acid with dilute sulfuric acid and distilled with steam until 2000 c.c. of distillate were collected. The volatile acids contained in the distillates were estimated by a Duclaux distillation. The figures obtained and also the average figures for two controls are given in Table 10.

The figures show that all but one of the cultures formed volatile acids. It is a significant fact that butyric and caproic acids were found to be present in approximately the proportion in which they occur in butter fat.⁶ This, together with the fact that the more active cultures gave very considerable quantities of insoluble acids, shows conclusively that this group of organisms can cause hydrolysis of the fat.

A small amount of acid of lower molecular weight than butyric was present in all the cultures. This has been reported as formic acid. Hart, Hastings, Flint, and Evans²⁰ found from 11.763 to 12.567 decinormal cubic centimeters of volatile acids in 300 c.c. of sterile milk incubated 4 months at 37 °C. They reported almost equivalent quantities of acetic and formic acids. Mixtures of these two acids so small in quantity as the figures in our experiments show, could not be identified with much certainty by the process of distillation employed. The

TABLE 10
VOLATILE ACIDS FROM 750 C.C. OF WHOLE MILK INOCULATED WITH *B. ABORTUS* VAR.
LIPOLYTICUS. RESULTS EXPRESSED IN N/10 C.C.

Acid	mc	hw	np	nj	nw	Control
Caproic.....	7.22	2.77	2.18	0.78	0.00	0.00
Butyric.....	13.74	6.65	5.90	2.36	1.06	1.01
Formic.....	3.62	11.74	2.95	4.69	3.11	4.35
Insoluble.....	3.73	0.50	0.00	0.00	0.00	0.00

cultures with one exception, *hw*, show nearly the same quantity of formic acid as the control. This would indicate that the acid was probably not produced by the bacteria but by some reaction during the sterilization or distillation. Therefore no special significance is attached to the formic acid figure.

The figures show in most cases only small amounts of butyric acid, and it required a growth of 2 weeks or more under optimal conditions of temperature to develop these small amounts. But since butyric acid possesses a very offensive odor, which can be detected in minute proportions of the acid, it was thought that perhaps the *B. abortus* group might have something to do with the development of undesirable flavors and odors in ripening cream. An earlier experiment had shown that lactic acid added to the milk to bring its acidity to 0.4% had no effect upon the multiplication of these organisms in the cream layer. This indicated that the by-products of organisms normally present in cream in large numbers do not check the growth of *B. abortus*.

A practical experiment was conducted to determine whether *B. abortus* var. *lipolyticus* might render cream undesirable under conditions similar to those to which cream is often subjected before use. The conditions were exaggerated in regard to the number of *B. abortus* present at the beginning of the experiment, altho on one or two occasions these organisms have been isolated from milk in numbers comparatively as high.

A very good quality of cream was mixed and then divided into 3 equal portions of 1 pint each. One flask of cream served as a control, and the other 2 were inoculated with cultures of the *B. abortus* type which had been isolated from milk. Culture *mc* used to inoculate one flask, was a typical culture of the *lipolyticus* variety.

Culture *sr*, which was used to inoculate the other flask, had been isolated from a "Special Milk" purchased in the market and supposed to be a good grade of raw milk. The lactose-agar plates made from this sample showed 78,000 colonies. The serum-agar plates showed 570,000 colonies per cubic centimeter. This indicated almost 500,000 of the *B. abortus* group per cubic centimeter. Culture *sr*, which represented these colonies, resembled the *B. abortus* type in morphology and general behavior. The manner of growth in serum agar, failure of growth in lactose agar, its morphology, and the fact that it was active in decomposing butter fat, indicated that it was related to the *B. abortus* type, and the finding of this strain in such large numbers in market milk gave a special interest to this organism in the experiment here recorded.

The three flasks of cream were allowed to stand in the room for about an hour, and then were placed in a thermostat at 17 C. where they were kept for 2 days, or until the end of the experiment. But they were removed twice to room temperature for a half hour, and once for 2 hours. This was to simulate the conditions to which cream might be subjected in a kitchen where no especial attention was given to its care.

TABLE 11
EFFECT OF *B. ABORTUS* ON FLAVOR OF CREAM

Judge	I Control Cream	II Cream Inoculated with MC	III Cream Inoculated with SR
24 HOURS AFTER INOCULATION			
A	Smoother in texture than others	Disagreeable odor. Taste not affected	Same as II
B	Good cream.....	An old, tho not bad, flavor	Same as I
C	Good cream.....	Slightly bad odor, but no difference in flavor	Same as II
D	Good cream.....	Bad odor, no difference in flavor	Same as I
E	Perfectly clean flav- or and odor	Bad odor and taste.....	Worse flavor than II
48 HOURS AFTER INOCULATION			
B	Mild odor.....	Bad odor, no difference in flavor	Same as II
C	By far the best.....	The worst of the three in odor. Flavor better than III	The worst of the three in taste, but odor not so bad as II
E	The best of the three	Not a good flavor.....	The worst of the three

Twenty-four hours after inoculation and again 48 hours after inoculation the creams were submitted to the judgment of several persons who knew nothing of the experiment. The flasks were relabeled before the second scoring. The opinions of the judges are arranged in tabular form in Table 11, which shows that after 24 hours the flask inoculated with Culture *mc*, which is typical of the strains isolated most frequently from milk, had acquired a disagreeable flavor and odor which was detected by all 5 judges. The flask inoculated with Culture *sr*, from market milk, was apparently not so bad, for only 3 of the 5 judges detected anything wrong with the cream. At the time of the 1st scoring all the creams were sweet. At the time of the 2nd scoring all 3 of the creams had soured. The creams which had been inoculated had large clots which could not be broken by violent shaking, and there had been a decided deterioration in the quality.

TABLE 12
BACTERIAL CONTENT OF CREAM INOCULATED WITH *BACILLUS ABORTUS*

	Control Cream		Cream Inoculated with MC		Cream Inoculated with SR	
	Total Number of Bacteria per c.c.	Number of B. Abortus per c.c.	Total Number of Bacteria per c.c.	Number of B. Abortus per c.c.	Total Number of Bacteria per c.c.	Number of B. Abortus per c.c.
At time of inoculation..	15,000	None isolated	280,000	265,000	1,500,000	1,200,000
After 24 hours.....	8,200,000	None isolated	12,500,000	2,000,000	12,000,000	None isolated
After 48 hours.....	540,000,000	None isolated	740,000,000	200,000,000	650,000,000	None isolated

Bacteriologic analyses of the creams just after inoculation and at the times of judging were made, the figures of which are given in Table 12. The figures showing the number of *B. abortus* are only rough approximations. The control cream had a low bacterial content, and if it contained *B. abortus* the number was so small that they failed to be isolated. The rapid multiplication of *mc*, in the presence of the normal flora of cream, shows what a previous experiment had indicated; namely, that the growth of *B. abortus* is not checked by the by-products of the other bacteria normally present in milk and cream. It shows also that *B. abortus* multiplies rapidly in cream that is subjected to temperatures at which it is commonly kept before use.

The inoculation of Culture *sr* was very heavy, but the other bacteria multiplied so much more rapidly that it failed to be isolated in the 2nd and 3rd analyses. There can be no doubt, however, that it exerted a deteriorating effect on the cream, for the appearance, odor, and flavor were affected, particularly on the second day, when this cream was unquestionably inferior to the control.

The results of this experiment show that *B. abortus* may be the cause of bad odors and flavors in ripened cream. The odor and flavor obtained after inoculation were such as are common in old cream.

B. Abortus in Mixed Market Milk.—Since *B. abortus* was found in samples of milk from individual cows of all 5 herds from which samples were taken, it was to be expected that these organisms could be isolated from mixed market milk, with low bacterial content. If the

bacterial content is very high, there might be large numbers of *B. abortus* present, but they would fail to be isolated. Therefore, the mixed market milks examined, were ascertained to be of good quality. The finding of organisms represented by Culture *sr* in numbers of about 500,000 per cubic centimeter in a good grade of market milk has already been mentioned. No *B. abortus* could be isolated from the cream used in the experiment recorded here, but it had been isolated 2 weeks earlier from mixed whole milk produced at the farm from which this cream came. Fewer than 200 abortion bacilli per cubic centimeter appeared in this milk.

Two bottles of certified milk were purchased in market at Chicago, kept at room temperature for about 6 hours, and then plated on serum agar. About 28,000 of the abortion bacilli were shown to be present in the cream layer of one sample and about 1900 in the cream layer of the other sample. In both samples the number of *B. abortus* was about 25% of the whole number of bacteria. The abortion bacilli can therefore be demonstrated in mixed milk.

Persistence of B. Abortus in Cows' Udders.—The milks of 4 of the cows of Dairy 1, which had contained *B. abortus*, were examined a 2nd time more than a year after the 1st examination. The samples were taken from the same quarter of the udder for both examinations. The data are given in Table 13. In the case of the two cows which gave milk containing only a few abortion bacilli at the time of the 1st examination, none of these organisms could be isolated from the milk in the 2nd examination. Cow 201, which gave 50,000 abortion bacilli per cubic centimeter at the time of the 1st examination, continued to give them in very large numbers. Cow 204 also continued to give these organisms in her milk in considerable numbers.

CONCLUSIONS

It appears from the study here reported that there is a definite udder flora comprising bacteria which belong to parasitic types. It is not surprising that the majority of udder bacteria should be of the same type as those common on the skin and mucous membrane of man and animals. The majority of the staphylococci on the skin are of the non-virulent variety which fails to produce pigment and fails to ferment mannite. But pathogenic varieties also are found on the skin, where they ordinarily cause no trouble. Similarly, the majority of the staphylococci of the udder are non-virulent, but varieties which

are capable of causing death when inoculated into experimental animals occasionally establish themselves in healthy udders. Whatever the variety may be, conditions in the udder are favorable to multiplication, and frequently large numbers are eliminated in the milk.

The pathogenic properties of the streptococci and bacilli common in milk when it leaves the udder are not discussed in this paper, but they, also, are parasitic in their nature.

When a bacterial culture is tested for its pathogenic properties, the body tissues and fluids are exposed directly to the toxins of the culture in question. When organisms enter into the digestive tract with the food the circumstances are different, for the body tissues and fluids are protected by the mucous membranes against the ravages of the bacteria that enter with the food. Therefore it cannot be

TABLE 13
PERSISTENCE OF *B. ABORTUS* IN COWS' UDDERS

Cow	Quarter	First Examination		Time Between 1st and 2nd Exam- inations	Second Examination	
		Total Bac- terial Count per c.c.	Total <i>B. abortus</i> per c.c.		Total Bac- terial Count per c.c.	Total <i>B. abortus</i> per c.c.
14	RB	750	Estimation impossible	19 months	230	None
18	RF	130	50	13 months	50	None
201	RF	50,000	50,000	13 months	10,600	10,000
204	RF	5600	5000	13 months	850	850

assumed that bacteria which are pathogenic to inoculated laboratory animals would be injurious to human beings when present in the milk consumed. It is a subject worthy of investigation. But since the bacteria of the udder are parasitic in their nature, and since pathogenic varieties are sometimes eliminated in considerable numbers from healthy udders, the data here reported add evidence to the growing conviction that all milk is safer for consumption after it has been pasteurized.

SUMMARY

One hundred and ninety-two samples of milk from 161 cows of 5 different dairies in 2 widely distant sections of the country have been studied. No consideration was given to those types of bacteria which occurred in the udder in small numbers. All the cultures which were studied in detail and included in this report represent bacteria which were multiplying in the udder and were found in the milk in considerable numbers.

TABLE 14
CHARACTERISTICS OF THE STAPHYLOCOCCI IN FRESHLY DRAWN MILK

Culture	Dairy	Cow	Action in Milk	Dex- trose	Lac- tose	Mal- tose	Raffi- nose	Man- nite
GROUP A								
ab	1	1	Curdled in 4 days.....	4.60	3.65	3.45	0.30	0.75
ak	1	2	Curdled in 4 days.....	4.75	4.35	2.85	0.20	1.35
cg	1	23	No change in 14 days.....	3.50	3.35	3.40	0.40	0.80
cn	1	23	Acid, not curdled, in 14 days..	1.10	1.05	1.60	0.25	0.65
cq	1	25	Curdled in 5 days.....	2.60	2.10	2.55	0.40	0.85
cs	1	100	Acid, not curdled, in 14 days..	3.25	3.15	3.20	-0.05	0.85
dc	1	27	Acid, not curdled, in 14 days..	2.75	3.30	2.55	0.35	1.25
dq	1	13	Acid, not curdled, in 14 days..	4.30	3.15	3.45	0.15	1.30
dr	1	13	Acid, not curdled, in 14 days..	4.70	3.60	3.50	0.10	1.25
ds	1	14	Curdled in 5 days.....	5.00	4.90	3.15	0.15	1.05
ea	1	7	Curdled in 14 days.....	3.75	4.30	3.05	0.05	1.25
fj	1	204	Acid, not curdled, in 14 days..	4.05	3.80	3.25	0.15	1.35
in	1	27	Curdled in 10 days.....	4.20	5.00	2.90	0.05	1.35
is	1	204	No change in 14 days.....	3.65	3.75	3.05	0.05	0.75
jm	1	2	Slight acidity in 14 days.....	3.55	3.25	0.70	0.10	1.00
kb	1	12	Curdled in 11 days.....	4.00	3.70	1.65	0.05	0.90
kn	1	12	Curdled in 11 days.....	4.05	4.00	1.75	0.15	2.05
kz	2	10051	Acid, not curdled, in 14 days..	3.15	3.50	4.10	0.05	1.15
lb	2	10039	Acid, not curdled, in 14 days..	3.60	3.25	3.80	0.06	1.15
lm	2	10006	Acid, not curdled, in 14 days..	3.25	3.55	3.95	0.10	0.60
lx	2	14759	Acid, not curdled, in 14 days..	3.95	3.45	2.20	0.10	1.30
mj	3	152	No change in 14 days.....	3.85	2.70	3.10	-0.05	1.25
ml	2	52	No change in 14 days.....	3.20	1.85	2.45	0.15	1.50
nf	4	304	Acid, not curdled, in 14 days..	3.75	3.50	3.25	0.10	1.30
nh	4	319	Curdled in 4 days.....	3.25	2.30	2.90	0.20	1.85
ni	4	330	Curdled in 4 days.....	3.75	3.00	2.80	0.25	1.60
ol	5	8	Acid, not curdled, in 14 days..	1.10	3.85	1.85	-0.20	0.80
ou	5	11	Acid, not curdled, in 14 days..	1.00	2.80	1.90	0.15	1.75
px	5	30	Acid, not curdled, in 14 days..	4.00	3.20	2.45	0.00	1.00
pz	5	31	No change in 14 days.....	3.85	3.00	2.30	0.00	1.55
qq	5	39	Acid, not curdled, in 14 days..	3.15	3.35	2.50	-0.15	0.85
qy	5	42	No change in 14 days.....	3.05	2.75	2.30	-0.15	1.35
rf	5	53	No change in 14 days.....	4.20	3.55	2.25	0.00	1.25
rj	5	55	Acid, not curdled, in 14 days..	4.60	3.70	2.15	-0.45	1.20
rq	5	56	Acid, not curdled, in 14 days..	4.20	3.55	2.50	-0.20	1.50
rv	5	58	Acid, not curdled, in 14 days..	3.40	2.95	2.35	0.20	0.85
rz	5	59	Acid, not curdled, in 14 days..	4.25	3.80	1.55	-0.10	1.75
GROUP B								
an	1	2	Curdled in 4 days.....	4.00	2.00	3.25	0.30	0.90
ap	1	2	Curdled in 2 days.....	3.60	1.70	3.30	0.35	1.50
bn	1	3	Curdled in 4 days.....	4.90	1.85	3.20	0.25	1.65
dx	1	12	Acid, not curdled, in 14 days..	3.90	3.90	3.30	0.40	2.25
dy	1	4	No change in 14 days.....	3.80	3.50	3.45	0.00	1.10
eb	1	9	Curdled in 4 days.....	4.00	4.20	3.65	0.20	3.20
fd	1	16	Acid, not curdled, in 14 days..	4.15	4.15	3.15	-0.05	1.30
fe	1	16	Acid, not curdled, in 14 days..	3.65	3.80	3.65	0.10	2.25
fg	1	21	Acid, not curdled, in 14 days..	4.30	4.30	2.90	0.00	1.15
fl	1	205	Acid, not curdled, in 14 days..	4.65	3.90	3.55	0.10	1.05
fs	1	21	Acid, not curdled, in 14 days..	3.20	1.15	3.50	0.10	1.35
fw	1	24	Acid, not curdled, in 14 days..	3.25	2.90	2.75	0.25	1.00
ia	1	16	Acid, not curdled, in 14 days..	3.40	4.10	3.15	-0.15	3.25
ib	1	16	Acid, not curdled, in 14 days..	3.30	3.10	4.20	-0.10	3.10
ji	1	21	Curdled in 4 days.....	3.40	2.40	1.40	0.25	1.10
im	1	27	Curdled in 10 days.....	3.75	4.55	3.20	-0.10	1.30
io	1	27	Curdled in 10 days.....	4.20	5.90	2.60	0.10	1.65
ip	1	27	Curdled in 10 days.....	3.90	3.45	2.65	0.05	1.55
jo	1	2	Curdled in 11 days.....	3.35	2.85	1.80	0.25	0.80
nq	4	289	Curdled in 4 days.....	3.25	3.20	4.15	0.40	1.85
rk	5	55	Curdled in 10 days.....	5.65	3.20	2.45	-0.40	2.25
rr	5	56	Curdled in 14 days.....	3.10	3.20	2.20	-0.15	1.95
so	5	59	Curdled in 14 days.....	3.35	3.05	2.05	-0.05	1.75
sf	5	68	Curdled in 14 days.....	3.50	3.30	1.65	-0.10	1.35
sm	5	75	Curdled in 14 days.....	3.25	3.00	2.05	0.00	2.10

TABLE 14—Continued

CHARACTERISTICS OF THE STAPHYLOCOCCI IN FRESHLY DRAWN MILK

Glycerin	Hemolysis	Liquefaction of Gelatin	Reduction of Nitrates	Decomposition of Asparagin	Decomposition of Urea	Chromogenesis
GROUP A—Continued						
0.10	+++	30 mm.	—	—	+	19 YOY, light cadmium
1.35	+++	20 mm.	—	—	+	19 OY d, buff yellow
0.45	+++	8 mm.	—	—	+	19 YOY, light cadmium
0.20	—	6 mm.	—	—	—	Slight pigment
0.35	++	6 mm.	—	—	+	17 OY f, pale yellow orange
0.30	+++	10 mm.	—	—	+	19 YOY, light cadmium
0.30	—	0 mm.	—	—	—	23" Y f, marguerite yellow
0.45	+++++	11 mm.	—	—	+	17 OY, cadmium yellow
0.30	+++	8 mm.	—	—	+	17 OY, cadmium yellow
0.45	+++	18 mm.	—	—	+	19 YOY d, buff yellow
0.45	+++	17 mm.	—	—	+	19 YOY, light cadmium
0.25	+++	13 mm.	—	—	+	17 OY, cadmium yellow
0.35	+++	17 mm.	—	—	—	19 YOY b, apricot yellow
-0.05	+++	Slight	—	—	—	17 OY, cadmium yellow
-0.15	+++	6 mm.	—	—	—	19 YOY b, apricot yellow
0.20	+++	18 mm.	—	—	—	19 YOY, light cadmium
0.20	+++	16 mm.	—	—	—	19 YOY b, apricot yellow
0.05	+++	0 mm.	—	—	+	17 OY, cadmium yellow
0.40	+++	0 mm.	—	—	+	17 OY, cadmium yellow
0.30	+++	0 mm.	—	—	+	19 YOY b, apricot yellow
0.25	+++	0 mm.	—	—	+	17 OY b, deep chrome
0.35	++	8 mm.	—	—	+	15 YO, orange
0.45	+	0 mm.	—	—	+	17 OY, cadmium yellow
0.30	+++	0 mm.	—	—	+	17 OY b, deep chrome
0.20	+++++	20 mm.	—	—	+	17 OY b, deep chrome
0.25	+++++	28 mm.	—	—	—	17 OY, cadmium yellow
0.20	+++	25 mm.	—	—	—	17 OY, cadmium yellow
0.45	+++	24 mm.	+	—	+	19" YOY f, cartridge buff
0.45	+++	25 mm.	—	—	—	17 OY, cadmium yellow
0.30	++	10 mm.	—	—	+	19 YOY, light cadmium
0.25	+	15 mm.	+	—	+	15 YO d, orange buff
0.35	+++	0 mm.	—	—	+	15 YO d, orange buff
0.35	+	15 mm.	—	—	+	17 OY b, deep chrome
0.30	—	27 mm.	—	—	—	17 OY, cadmium yellow
0.45	+++	27 mm.	—	—	—	17 OY, cadmium yellow
0.30	+	0 mm.	—	—	+	17 OY f, pale orange yellow
0.40	+++	0 mm.	—	—	—	17" OY b, antimony yellow
GROUP B—Continued						
1.15	+	12 mm.	—	—	+	17 OY b, deep chrome
1.80	—	7 mm.	—	—	+	15 YO b, capucine yellow
0.90	+	16 mm.	+	—	+	19 YOY f, maize yellow
0.65	++++	20 mm.	—	—	+	17 OY b, deep chrome
0.65	+++	28 mm.	—	—	+	17 OY, cadmium yellow
1.55	+++	21 mm.	—	—	+	17 OY, cadmium yellow
0.60	+++	17 mm.	—	—	+	17 OY b, deep chrome
1.10	+++	16 mm.	—	—	+	19" YOY f, cream color
0.60	+++	14 mm.	—	—	+	19 YOY, light cadmium
0.55	+++	15 mm.	—	—	+	15 YOY d, orange
0.70	—	15 mm.	—	—	+	19 YOY d, buff yellow
0.80	—	10 mm.	—	—	+	19 YOY d, buff yellow
1.05	+++++	16 mm.	+	—	+	19" YOY, honey yellow
1.70	+++++	27 mm.	+	—	+	19" YOY, honey yellow
0.75	++	15 mm.	+	—	+	15 YO b, capucine yellow
0.70	+++	20 mm.	—	—	—	17 OY b, deep chrome
0.55	+++	16 mm.	—	—	—	19 YOY d, buff yellow
0.60	+++	18 mm.	—	—	—	17 OY b, deep chrome
0.60	+++	14 mm.	—	—	—	19 YOY, light cadmium
0.85+	+++	25 mm.	—	—	+	17 OY b, deep chrome
0.90	+++	31 mm.	—	—	+	15 YO d, orange buff
0.70	—	18 mm.	—	—	—	19" YOY f, cartridge buff
1.65	—	20 mm.	—	—	—	19" YOY f, cartridge buff
0.70	—	25 mm.	—	—	—	19" YOY f, cartridge buff
2.20	—	25 mm.	+	—	—	19" YOY f, cartridge buff

TABLE 14—*Continued*
 CHARACTERISTICS OF THE STAPHYLOCOCCI IN FRESHLY DRAWN MILK

Culture	Dairy	Cow	Action in Milk	Dex- trose	Lac- tose	Mal- tose	Raffi- nose	Man- nite
GROUP C								
ac	1	1	Curdled in 5 days.....	4.20	1.20	2.95	0.50	0.90
ar	1	3	Curdled in 5 days.....	3.65	2.00	2.65	0.50	1.20
bb	1	2	Curdled in 4 days.....	3.75	2.30	3.05	0.75	1.30
be	1	2	Curdled in 5 days.....	4.95	4.20	2.15	0.55	1.20
bw	1	23	Curdled in 5 days.....	4.00	1.90	3.25	0.65	1.85
ck	1	21	Curdled in 4 days.....	2.60	1.55	2.25	0.65	2.35
cl	1	21	Acid, not curdled, in 14 days..	2.15	3.80	2.80	1.70	1.20
cx	1	19	Acid, not curdled, in 14 days..	1.95	2.35	2.90	1.20	1.15
da	1	26	Acid, not curdled, in 14 days..	1.40	2.15	3.15	0.55	0.55
ol	5	1	Acid, not curdled, in 14 days..	1.45	2.40	2.35	1.15	0.90
op	5	9	Acid, not curdled, in 14 days..	2.85	0.75	2.15	1.00	0.90
po	5	23	Curdled in 5 days.....	1.60	3.10	2.50	1.35	2.05
GROUP D								
aj	1	2	Curdled in 4 days.....	3.75	1.50	3.30	0.85	1.25
az	1	2	Curdled in 4 days.....	4.40	1.55	2.80	0.80	1.35
ba	1	2	Curdled in 5 days.....	3.55	1.35	2.90	0.55	1.30
bd	1	1	Curdled in 4 days.....	4.50	1.05	2.70	0.50	0.60
bo	1	3	Curdled in 4 days.....	1.95	2.25	3.05	0.65	1.40
bx	1	23	Curdled in 5 days.....	2.95	1.85	2.90	0.50	1.40
cc	1	100	Curdled in 5 days.....	2.65	2.05	2.80	1.40	0.65
cd	1	100	Curdled in 5 days.....	2.95	2.45	3.05	1.00	1.50
ci	1	17	Curdled in 5 days.....	2.90	2.15	2.55	0.90	1.45
cm	1	21	Curdled in 5 days.....	3.90	1.45	3.15	0.90	1.75
co	1	23	Curdled in 5 days.....	2.75	1.00	2.95	0.75	1.30
cr	1	27	Acid, not curdled, in 14 days..	1.40	2.05	2.90	1.00	1.15
ct	1	100	Curdled in 5 days.....	2.50	1.70	3.20	0.75	1.90
df	1	19	Acid, not curdled, in 14 days..	1.85	1.80	0.85	1.35	2.10
dm	1	7	Acid, not curdled, in 14 days..	4.85	4.70	2.70	0.50	1.35
nl	4	62	No change in 14 days.....	2.95	1.60	2.20	1.45	1.30
of	5	1	No change in 14 days.....	3.15	2.90	1.95	1.85	1.35
qv	5	39	Curdled in 10 days.....	3.55	2.40	2.15	1.05	1.50
rb	5	46	Acid, not curdled, in 14 days..	2.85	2.20	2.35	0.80	0.95
ru	5	58	Acid, not curdled, in 14 days..	1.05	2.90	2.20	2.45	0.65
GROUP E								
nx	4	160	Acid, not curdled, in 14 days..	5.45	3.10	2.05	3.90	0.05
oh	5	1	Acid, not curdled, in 14 days..	1.25	3.25	3.05	1.40	0.05
GROUP F								
aa	1	1	Curdled in 2 days.....	5.20	4.20	4.50	0.35	0.00
am	1	2	Acid, not curdled, in 14 days..	5.25	3.70	5.20	0.20	-0.40
at	1	14	Curdled in 2 days.....	4.60	3.95	4.10	0.20	0.25
aw	1	14	Curdled in 4 days.....	4.85	4.05	4.15	0.35	0.15
ax	1	14	Curdled in 2 days.....	4.75	3.80	4.30	0.40	-0.05
ay	1	2	Curdled in 4 days.....	5.20	4.60	4.45	0.25	-0.20
bh	1	1	Curdled in 4 days.....	4.45	4.25	4.35	0.20	-0.15
bi	1	14	Curdled in 4 days.....	4.90	3.60	4.24	0.25	0.05
du	1	9	Curdled in 5 days.....	4.30	4.50	4.20	0.05	0.20
dv	1	13	Curdled in 7 days.....	4.80	4.40	4.05	0.10	0.30
ef	1	9	Curdled in 6 days.....	4.40	3.90	3.90	0.25	0.15
eh	1	10	Curdled in 6 days.....	3.65	4.00	4.15	0.15	0.20
ej	1	13	Curdled in 6 days.....	4.30	3.80	4.10	0.25	-0.15
fq	1	19	Curdled in 6 days.....	4.35	3.90	4.00	0.10	-0.05
fr	1	19	Curdled in 7 days.....	4.35	3.60	4.00	0.15	-0.15
jn	1	2	Curdled in 8 days.....	4.50	4.10	3.25	0.10	0.15
jr	1	8	Curdled in 6 days.....	3.45	3.45	3.90	0.00	-0.05

TABLE 14—*Continued*
CHARACTERISTICS OF THE STAPHYLOCOCCI IN FRESHLY DRAWN MILK

Glyc- erin	Hemoly- sis	Lique- faction of Gelatin	Reduc- tion of Nitrates	Decom- position of As- paragin	Decom- position of Urea	Chromogenesis
GROUP C— <i>Continued</i>						
0.15	+	10 mm.	+	—	+	17 OY f, pale orange yellow
0.25	+++	Slight	+	—	+	15 YO b, capucine yellow
0.50	++	Slight	+	—	+	15 YO b, capucine yellow
0.35	+++	6 mm.	—	—	+	19 YOY d, buff yellow
0.50	+	15 mm.	—	—	+	17 OY d, light orange yellow
0.45	+++	12 mm.	—	—	+	15 YO f, pale yellow orange
0.45	—	Slight	—	—	+	17 OY f, pale orange yellow
0.30	—	0 mm.	—	—	—	19 YOY, light cadmium
0.35	+	2 mm.	—	—	+	19 YOY d, buff yellow
0.40	+	12 mm.	—	—	+	19 YOY d, buff yellow
0.15	—	12 mm.	—	—	+	15 YO d, orange buff
0.40	++++	25 mm.	—	—	+	17" OY b, antimony yellow
GROUP D— <i>Continued</i>						
1.45	++	12 mm.	+	—	+	15 YO b, capucine yellow
0.75	+	8 mm.	+	—	+	15 YO b, capucine yellow
0.65	+	10 mm.	—	—	+	15 YO b, capucine yellow
0.60	—	Slight	—	—	+	15 YO d, orange buff
0.55	—	Slight	+	—	+	15 YO b, capucine yellow
0.80	+	9 mm.	+	—	+	17 OY b, deep chrome
0.55	+	15 mm.	—	—	+	17 OY b, deep chrome
0.65	+++	15 mm.	+	—	+	17 OY d, light orange yellow
0.80	+++	17 mm.	—	—	+	17 OY f, pale orange yellow
0.65	++	15 mm.	—	—	+	15 YO f, pale yellow orange
0.65	+	Slight	—	—	+	15 YO d, orange buff
0.80	+	18 mm.	—	—	—	17 OY b, deep chrome
0.65	+++	13 mm.	+	—	+	17 OY f, pale orange yellow
1.70	+++	11 mm.	—	—	—	17 OY b, deep chrome
0.55	+++	16 mm.	—	—	+	19 YO y, light cadmium
0.70	++	15 mm.	—	—	+	17 OY b, deep chrome
0.80	—	15 mm.	—	—	+	15 YO b, capucine yellow
0.80	+	20 mm.	—	—	+	15 YO d, orange buff
0.90	—	15 mm.	—	—	+	15 YO d, orange buff
1.65	+	17 mm.	+	—	+	19" YOY d, naples yellow
GROUP E— <i>Continued</i>						
0.00	+	0 mm.	—	—	—	Seant growth
-0.05	—	3 mm.	—	—	+	21" OYY f, ivory yellow
GROUP F— <i>Continued</i>						
0.70	++	10 mm.	—	—	+	Slight pigment
0.95	—	0 mm.	—	—	—	21" OYY f, ivory yellow
0.80	—	7 mm.	—	—	—	21" OYY f, ivory yellow
0.90	+	3 mm.	—	—	+	21" OYY f, ivory yellow
0.90	—	6 mm.	—	—	+	21" OYY f, ivory yellow
0.80	+	Slight	—	—	+	21" OYY f, ivory yellow
1.35	+++	5 mm.	—	—	+	21" OYY f, ivory yellow
0.85	—	2 mm.	—	—	+	21" OYY f, ivory yellow
0.85	+	13 mm.	—	—	+	19" YOY f, cream color
1.05	+	9 mm.	—	—	+	21" OYY f, ivory yellow
0.75	+	12 mm.	—	—	+	21" OYY f, ivory yellow
1.25	+	14 mm.	—	—	+	21" OYY f, ivory yellow
1.15	—	11 mm.	—	—	+	21" OYY f, ivory yellow
1.05	—	11 mm.	+	—	+	19" YOY f, cream color
1.10	—	11 mm.	—	—	+	19" YOY f, cream color
1.15	+++	6 mm.	—	—	—	19" YOY f, cream color
1.30	+++	5 mm.	—	—	—	19" YY f, cartridge buff

TABLE 14—*Continued*
CHARACTERISTICS OF THE STAPHYLOCOCCI IN FRESHLY DRAWN MILK

Culture	Dairy	Cow	Action in Milk	Dex- trose	Lac- tose	Mal- tose	Raffi- nose	Man- nite
GROUP F— <i>Continued</i>								
kx	2	14721	Curdled in 6 days.....	4.25	3.10	5.40	0.15	0.25
ky	2	73	Curdled in 6 days.....	3.00	3.40	5.40	0.15	—0.20
la	2	8	Curdled in 13 days.....	2.70	3.15	3.40	0.05	—0.15
ld	2	5	Curdled in 6 days.....	4.25	3.15	4.00	0.15	—0.10
lf	2	24125	Curdled in 4 days.....	4.35	3.50	3.30	0.20	—0.00
lj	2	10040	Acid, not curdled, in 14 days..	3.40	3.35	3.30	—0.05	0.00
lk	2	20428	Curdled in 6 days.....	3.25	3.20	5.40	0.00	0.00
lo	2	11219	Acid, not curdled, in 14 days..	3.85	3.05	5.20	0.05	—0.10
lp	2	14776	Curdled in 6 days.....	4.00	3.30	4.95	0.15	—0.15
lq	2	16976	Curdled in 4 days.....	3.95	3.55	5.15	0.10	0.05
lu	2	10322	Curdled in 6 days.....	4.05	2.95	5.30	0.10	—0.10
lv	2	7563	Curdled in 4 days.....	4.20	3.10	5.50	—0.20	0.00
ly	2	7563	Curdled in 6 days.....	4.45	3.90	2.95	0.25	—0.05
lz	3	454	Curdled in 2 days.....	3.65	2.95	4.90	0.05	0.05
ma	3	454	Curdled in 6 days.....	3.05	3.10	3.90	0.20	—0.10
mf	3	319	Curdled in 13 days.....	3.95	2.90	4.20	0.25	—0.05
mo	3	278	Curdled in 13 days.....	3.35	3.20	4.70	0.10	—0.20
mq	3	361	Curdled in 13 days.....	3.45	2.80	4.45	0.25	0.30
my	4	335	Acid, not curdled, in 14 days..	3.15	2.60	5.80	0.35	0.10
nd	4	304	Curdled in 6 days.....	3.95	3.45	3.20	—0.05	—0.15
ng	4	315	Curdled in 12 days.....	4.45	3.00	3.45	0.05	—0.10
ny	4	180	Curdled in 6 days.....	4.05	3.10	3.90	0.25	0.05
ob	4	62	Curdled in 4 days.....	4.40	3.25	4.20	0.10	0.15
oc	4	372	Curdled in 6 days.....	3.10	3.05	4.50	—0.05	—0.40
pa	5	12	Curdled in 7 days.....	1.15	3.40	2.90	—0.10	0.05
pe	5	18	Curdled in 7 days.....	4.00	3.80	2.80	0.15	0.20
pe	5	18	Curdled in 10 days.....	1.20	3.50	3.00	0.00	0.10
pi	5	20	Curdled in 9 days.....	3.80	3.65	3.70	0.20	0.25
pi	5	21	Curdled in 7 days.....	4.95	3.15	2.70	0.00	0.10
pq	5	24	Curdled in 8 days.....	1.05	3.35	2.90	0.05	0.10
qb	5	31	Curdled in 7 days.....	4.55	3.05	2.60	—0.10	0.00
qd	5	31	Curdled in 7 days.....	1.00	3.20	2.85	0.10	0.10
qg	5	34	Curdled in 7 days.....	1.50	3.30	2.75	—0.25	0.15
qh	5	34	Curdled in 10 days.....	4.55	3.85	2.80	—0.10	0.20
qp	5	36	Curdled in 8 days.....	1.20	3.60	2.55	—0.15	—0.10
ra	5	42	Curdled in 7 days.....	1.00	3.30	2.60	—0.05	0.20
rd	5	46	Curdled in 10 days.....	4.70	4.00	2.45	—0.05	0.10
re	5	48	Curdled in 7 days.....	1.05	3.15	2.80	0.05	0.20
sa	5	59	Curdled in 11 days.....	4.80	3.95	2.70	0.00	0.20
sj	5	73	Curdled in 9 days.....	1.40	3.10	2.60	0.05	0.10
GROUP G								
bt	1	19	Curdled in 14 days.....	3.70	4.30	3.25	0.15	0.10
bu	1	19	Curdled in 14 days.....	3.90	3.95	2.55	0.00	0.10
bv	1	19	Curdled in 14 days.....	4.15	4.00	3.10	0.30	0.25
ig	1	19	Curdled in 6 days.....	4.10	4.30	3.55	0.20	0.15
iz	1	8	Curdled in 11 days.....	3.85	3.50	3.70	0.25	—0.05
kx	2	14721	Curdled in 6 days.....	4.25	3.10	5.40	0.15	0.25
lh	2	14758	Curdled in 6 days.....	2.85	2.65	2.60	0.25	0.15
ll	2	11814	Curdled in 13 days.....	4.00	2.75	5.20	—0.05	0.30
lo	2	11219	Acid, not curdled, in 13 days..	3.85	3.05	5.20	0.05	—0.10
lw	2	20422	Curdled in 13 days.....	3.95	3.65	4.90	0.15	—0.05
mb	3	454	Curdled in 5 days.....	3.80	3.15	2.10	0.20	0.05
md	3	060	Curdled in 6 days.....	3.65	2.90	4.90	0.30	0.00
mh	3	055	Acid, not curdled, in 14 days..	1.70	3.40	2.50	0.00	—0.10
mk	3	4	Curdled in 4 days.....	3.40	3.05	3.00	0.10	0.05
mr	3	349	Acid, not curdled, in 14 days..	3.55	3.15	1.45	0.25	0.05
ne	4	304	Acid, not curdled, in 14 days..	1.90	2.95	1.35	0.05	—0.10
oh	5	1	Acid, not curdled, in 14 days..	1.25	3.25	3.05	0.35	0.05
ph	5	20	Acid, not curdled, in 14 days..	3.65	3.65	2.20	—0.10	0.20
pm	5	21	Acid, not curdled, in 14 days..	4.20	3.60	1.40	0.05	0.15
pp	5	23	Curdled in 7 days.....	1.05	3.30	2.90	0.15	0.15
qo	5	36	Curdled in 7 days.....	1.20	2.25	3.50	0.00	0.25

TABLE 14—*Continued*
CHARACTERISTICS OF THE STAPHYLOCOCCI IN FRESHLY DRAWN MILK

Glycerin	Hemolysis	Liquefaction of Gelatin	Reduction of Nitrates	Decomposition of Asparagin	Decomposition of Urea	Chromogenesis
GROUP F— <i>Continued</i>						
0.85	—	16 mm.	—	—	+	19' YOY f, cream color
1.05	+	10 mm.	—	—	+	19" YOY d, cream buff
1.10	+	10 mm.	—	—	+	17 OY, cadmium yellow
0.95	—	12 mm.	—	—	+	19" YOY d, cream buff
1.25	++	15 mm.	—	—	—	19' YOY f, cream color
1.05	—	4 mm.	—	—	+	19' YOY f, cream color
1.30	++	16 mm.	—	—	+	19' YOY f, cream color
0.70	+	0 mm.	—	—	+	19' YOY f, cream color
1.00	+	10 mm.	—	—	—	17" OY d, pinkish buff
1.10	+	11 mm.	—	—	—	17 OY, cadmium yellow
1.15	+	11 mm.	—	—	+	19" YOY f, cartridge buff
1.75	—	10 mm.	—	—	+	13 OY o, cadmium orange
1.95	—	13 mm.	—	—	+	19" YOY d, cream buff
1.50	+	10 mm.	—	—	+	17 OY, cadmium yellow
1.25	+	10 mm.	—	—	—	19" YOY f, cartridge buff
1.60	+	16 mm.	—	—	—	19" YOY f, cartridge buff
1.10	+	13 mm.	—	—	+	19" YOY d, cream buff
1.15	+	13 mm.	—	—	—	19" YOY d, cream buff
0.80	++	0 mm.	—	—	—	23" Yellow f, marguerite yellow
0.75	+	3 mm.	—	—	+	13 OYO, cadmium orange
1.15	+	11 mm.	—	—	+	21" OYY f, ivory yellow
0.60	—	10 mm.	—	—	+	19" YOY d, cream buff
2.10	+	25 mm.	—	—	+	13 OYO cadmium orange
2.05	++	11 mm.	—	—	+	19' YOY f, cream color
1.20	+	17 mm.	—	—	—	19" YOY f, cartridge buff
1.10	+++	15 mm.	—	—	—	15 YO, orange
2.30	+	15 mm.	—	—	—	21" OYY f, ivory yellow
0.85	++	11 mm.	—	—	—	15 YO, orange
1.35	+	16 mm.	—	—	+	17 OY f, light buff
1.30	+++	15 mm.	—	—	—	17" OY d, vinaceous buff
1.75	++++	14 mm.	—	—	+	19' YOY f, cream color
1.15	+++	15 mm.	—	—	—	19" YOY f, cartridge buff
1.50	+++	15 mm.	—	—	—	21" OYY f, ivory yellow
2.20	+++	14 mm.	—	—	—	13 OYO, cadmium orange
2.45	+	15 mm.	—	—	+	13 OYO, cadmium orange
1.35	+	15 mm.	—	—	—	19" YOY f, cartridge buff
3.00	+	15 mm.	—	—	+	15 YO, orange
1.35	—	13 mm.	—	—	—	21" OYY f, ivory yellow
1.45	+	15 mm.	—	—	+	13 OYO, cadmium orange
2.50	+	14 mm.	—	—	+	13 OYO, cadmium orange
GROUP G— <i>Continued</i>						
—0.10	—	4 mm.	—	—	+	17 OY b, deep chrome
0.30	—	8 mm.	—	—	+	19' YOY f, cream color
0.30	—	4 mm.	—	—	+	19' YOY f, cream color
0.20	+	15 mm.	—	—	+	19' YOY f, cream color
0.30	+	10 mm.	—	—	—	21" OYY f, ivory yellow
0.50	—	16 mm.	—	—	+	19' YOY f, cream color
0.30	—	20 mm.	+	—	—	19" YOY b, chamois
0.00	—	0 mm.	—	—	+	19" YOY d, cream buff
0.45	+	0 mm.	—	—	+	19' YOY f, cream color
0.45	—	15 mm.	—	—	+	13 OY, cadmium orange
0.20	+++	15 mm.	—	—	+	23 Y f, Martius yellow
0.35	—	8 mm.	—	—	+	19' YOY f, cream color
0.15	+++	0 mm.	—	—	+	19 YOY, light cadmium
0.25	+++	15 mm.	—	—	+	23 Y, strontian yellow
0.30	+	25 mm.	—	—	+	15 YO, orange
—0.10	+	0 mm.	—	—	—	19 YOY b, apricot yellow
—0.05	—	3 mm.	—	—	+	21" OYY f, ivory yellow
0.45	—	20 mm.	—	—	—	17 OY d, light orange yellow
0.10	—	0 mm.	—	—	—	17 OY, cadmium yellow
0.35	—	14 mm.	—	—	+	21" OYY f, ivory yellow
0.35	+++	28 mm.	—	—	—	21 OYY d, pinard yellow

TABLE 14—*Continued*
CHARACTERISTICS OF THE STAPHYLOCOCCI IN FRESHLY DRAWN MILK

Culture	Dairy	Cow	Action in Milk	Dex- trose	Lac- tose	Mal- tose	Raffi- nose	Man- nite
GROUP G— <i>Continued</i>								
qt	5	39	Acid, not curdled, in 14 days..	3.95	2.85	2.20	0.30	0.10
qu	5	39	Acid, not curdled, in 14 days..	3.95	3.90	2.70	—0.05	0.16
ro	5	54	Acid, not curdled, in 14 days..	4.20	1.10	2.15	0.00	0.20
sh	5	73	Curdled in 14 days.....	1.10	3.30	1.60	0.10	0.20
sk	5	73	Curdled in 4 days.....	4.70	3.15	2.95	0.25	0.00
GROUP H								
as	1	14	Acid, not curdled, in 14 days..	4.00	3.30	0.35	0.15	0.05
fh	1	25	Acid, not curdled, in 14 days..	5.00	3.95	0.20	0.05	—0.05
fy	1	24	Acid, not curdled, in 14 days..	2.45	2.45	0.50	0.25	0.45
ik	1	25	No change in 14 days.....	4.60	5.05	0.05	0.00	0.35
nw	5	195	No change in 14 days.....	2.90	3.05	0.00	0.15	0.30

In 32, or 16.1% of the whole number of samples studied, bacteria were not multiplying in numbers worth considering.

Three types of bacteria were found commonly present in milk from all 5 dairies; they were streptococci, staphylococci, and bacilli.

The ordinary milk-souring organism, *Streptococcus lacticus* (Kruse), was not found in any of the samples of milk. It does not appear to localize and multiply in the udder.

Long-chained streptococci which failed to give the reduction of litmus in milk cultures characteristic for *Streptococcus lacticus* were isolated from 29, or 15.1% of the whole number. The highest number found per cubic centimeter was 264,000.

Micrococci were found in 113, or 58.8% of the whole number. The highest number found per cubic centimeter was 80,000.

The majority of the micrococci were shown to belong to one group, which agrees in characteristics with the pyogenic staphylococci. The majority of cultures of this type were non-virulent; some cultures were virulent; 2 cultures possessed such a high degree of virulence that inoculated rabbits died in about 16 hours.

Three other types of micrococci from the udder gave reactions which differed from those of the pyogenic staphylococci sufficiently to separate them into distinct groups. One of these groups is described, but no name is suggested for it because of the small number of cultures studied. Another group was identified with *M. luteus* according to

TABLE 14—*Continued*
CHARACTERISTICS OF THE STAPHYLOCOCCI IN FRESHLY DRAWN MILK

Glycerin	Hemolysis	Liquefaction of Gelatin	Reduction of Nitrates	Decomposition of Asparagin	Decomposition of Urea	Chromogenesis
GROUP G— <i>Continued</i>						
0.30	—	6 mm.	+	—	—	19 YOY f, cream color
0.30	+++	14 mm.	—	—	+	13 OYO, cadmium orange
0.10	—	26 mm.	—	—	—	15 YO d, orange buff
0.45	+++	24 mm.	—	—	+	21 OYY d, pinard yellow
0.15	—		—	—	—	Slight pigment
GROUP H— <i>Continued</i>						
0.40	+	0 mm.	—	—	—	21" OYY f, ivory yellow
0.45	—	12 mm.	+	—	+	23" Y f, marguerite yellow
0.00	+	0 mm.	—	—	—	Slight pigment
0.05	—	10 mm.	+	—	—	21" OYY f, ivory yellow
0.25	+	17 mm.	—	—	—	21" OYY f, ivory yellow

the Winslow's classification. The third group was characterized by the rapid and complete peptonization of milk. The name *Micrococcus caseolyticus* is suggested for this group.

Peculiar strains of the types of bacteria commonly present in freshly drawn milk were sometimes found localized in the udders of several cows of one dairy. A few cases were found of peculiar species, unlike any of the other udder organisms, being localized in the udders of several cows of one dairy.

The bacilli commonly present in milk from all 5 dairies were shown to be related to *B. abortus*. Three varieties of this type were distinguished.

The variety of *B. abortus* occurring most frequently in the samples of milk is designated *B. abortus* var. *lipolyticus* because it decomposes butter fat. In its cultural characteristics this variety agrees closely with Bang's original description of *B. abortus*. Cultures of this variety were shown to be capable of imparting undesirable flavors and odors to cream kept under conditions to which cream is frequently subjected.

Two other varieties of *B. abortus* type differed considerably from the *lipolyticus* variety, but resembled the cultures isolated from pathogenic sources and studied for comparison.

Cultures of *B. abortus* type were isolated from 45, or 23.4% of the 192 samples studied. The highest number of these bacilli found per cubic centimeter was 50,000.

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